

COMPOSITIONS AND METHODS FOR THE
THERAPY AND DIAGNOSIS OF LUNG CANCER

REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. Patent Application
5 No. 09/614,124, filed July 11, 2000, which is a continuation-in-part of U.S. Patent
Application No. 09/589,184, filed June 5, 2000, which is a continuation-in-part of U.S.
Patent Application No. 09/560,406, filed April 27, 2000, which is a continuation-in-part of
U.S. Patent Application No. 09/546,259, filed April 10, 2000, which is a continuation-in-
part of U.S. Patent Application No. 09/533,077, filed March 22, 2000, which is a
10 continuation-in-part of U.S. Patent Application No. 09/519,642 filed March 6, 2000, which
is a continuation-in-part of U.S. Patent Application No. 09/476,300, filed December 30,
1999, which is a continuation-in-part of U.S. Patent Application No. 09/466,867, filed
December 17, 1999, which is a continuation-in-part of U.S. Patent Application 09/419,356,
filed October 15, 1999, which is a continuation-in-part of U.S. Patent Application
15 No. 09/346,492, filed June 30, 1999, and is related to PCT/US00/18061, filed 6/30/00.

TECHNICAL FIELD OF THE INVENTION

The present invention relates generally to therapy and diagnosis of cancer,
such as lung cancer. The invention is more specifically related to polypeptides comprising
at least a portion of a lung tumor protein, and to polynucleotides encoding such
20 polypeptides. Such polypeptides and polynucleotides may be used in compositions for
prevention and treatment of lung cancer, and for the diagnosis and monitoring of such
cancers.

BACKGROUND OF THE INVENTION

Cancer is a significant health problem throughout the world. Although
25 advances have been made in detection and therapy of cancer, no vaccine or other
universally successful method for prevention or treatment is currently available. Current

therapies, which are generally based on a combination of chemotherapy or surgery and radiation, continue to prove inadequate in many patients.

Lung cancer is the primary cause of cancer death among both men and women in the U.S., with an estimated 172,000 new cases being reported in 1994. The five-
5 year survival rate among all lung cancer patients, regardless of the stage of disease at diagnosis, is only 13%. This contrasts with a five-year survival rate of 46% among cases detected while the disease is still localized. However, only 16% of lung cancers are discovered before the disease has spread.

Early detection is difficult since clinical symptoms are often not seen until
10 the disease has reached an advanced stage. Currently, diagnosis is aided by the use of chest x-rays, analysis of the type of cells contained in sputum and fiberoptic examination of the bronchial passages. Treatment regimens are determined by the type and stage of the cancer, and include surgery, radiation therapy and/or chemotherapy.

In spite of considerable research into therapies for this and other cancers,
15 lung cancer remains difficult to diagnose and treat effectively. Accordingly, there is a need in the art for improved methods for detecting and treating such cancers. The present invention fulfills these needs and further provides other related advantages.

SUMMARY OF THE INVENTION

Briefly stated, the present invention provides compositions and methods for
20 the diagnosis and therapy of cancer, such as lung cancer. In one aspect, the present invention provides polypeptides comprising at least a portion of a lung tumor protein, or a variant thereof. Certain portions and other variants are immunogenic, such that the ability of the variant to react with antigen-specific antisera is not substantially diminished. Within certain embodiments, the polypeptide comprises a sequence that is encoded by a
25 polynucleotide sequence selected from the group consisting of: (a) sequences recited in SEQ ID NO: 1, 11-13, 15, 20, 23-27, 29, 30, 33, 34, 39, 41, 43-46, 51, 52, 57, 58, 60, 62, 65-67, 69-71, 74, 76, 79, 80, 84, 86, 89-92, 95, 97, 98, 101, 110, 111, 113-119, 121-128, 130-134, 136, 138, 139, 141, 143, 146-151, 153, 154, 157-160, 162-164, 167-178, 180,

181, 183, 186-190, 192, 193, 195-220, 224, 226-231, 234, 236, 237, 240, 241, 244-246, 248, 254, 255, 261, 262, 266, 270, 275, 280, 282, 283, 288, 289, 290, 292, 295, 301, 303, 304, 309, 311, 341-782, 784, 785, 790, 792, 794, 796, 800-804, 807, 808 and 810-826, 1240, 1243, 1247, 1269, 1272, 1280, 1283, 1285, 1286, 1289, 1300, 1309, 1318, 1319, 5 1327, 1335, 1339, 1346, 1359, 1369, 1370, 1371, 1393, 1398, 1405, 1408, 1413, 1414, 1417, 1422, 1429, 1432, 1435, 1436, 1438-1442, 1447, 1450, 1453, 1463, 1467, 1470, 1473, 1475, 1482, 1486, 1491-1494, 1501, 1505, 1506, 1514-1517, 1520, 1522, 1524, 1535, 1538, 1542, 1543, 1547, 1554, 1557, 1559, 1561, 1563, and 1669; (b) variants of a sequence recited in SEQ ID NO: 1, 11-13, 15, 20, 23-27, 29, 30, 33, 34, 39, 41, 43-46, 51, 10 52, 57, 58, 60, 62, 65-67, 69-71, 74, 76, 79, 80, 84, 86, 89-92, 95, 97, 98, 101, 110, 111, 113-119, 121-128, 130-134, 136, 138, 139, 141, 143, 146-151, 153, 154, 157-160, 162-164, 167-178, 180, 181, 183, 186-190, 192, 193, 195-220, 224, 226-231, 234, 236, 237, 240, 241, 244-246, 248, 254, 255, 261, 262, 266, 270, 275, 280, 282, 283, 288, 289, 290, 292, 295, 301, 303, 304, 309, 311, 341-782, 784, 785, 790, 792, 794, 796, 800-804, 807, 15 808, 810-826, 1240, 1243, 1247, 1269, 1272, 1280, 1283, 1285, 1286, 1289, 1300, 1309, 1318, 1319, 1327, 1335, 1339, 1346, 1359, 1369, 1370, 1371, 1393, 1398, 1405, 1408, 1413, 1414, 1417, 1422, 1429, 1432, 1435, 1436, 1438-1442, 1447, 1450, 1453, 1463, 1467, 1470, 1473, 1475, 1482, 1486, 1491-1494, 1501, 1505, 1506, 1514-1517, 1520, 1522, 1524, 1535, 1538, 1542, 1543, 1547, 1554, 1557, 1559, 1561, 1563, and 1669; and 20 (c) complements of a sequence of (a) or (b). In specific embodiments, the polypeptides of the present invention comprise at least a portion of a tumor protein that includes an amino acid sequence selected from the group consisting of sequences recited in SEQ ID NO: 786, 787, 791, 793, 795, 797-799, 806, 809, 827, 1670-1675 and 1677-1678 and variants thereof.

25 The present invention further provides polynucleotides that encode a polypeptide as described above, or a portion thereof (such as a portion encoding at least 15 amino acid residues of a lung tumor protein), expression vectors comprising such polynucleotides and host cells transformed or transfected with such expression vectors.

Within other aspects, the present invention provides pharmaceutical compositions comprising a polypeptide or polynucleotide as described above and a physiologically acceptable carrier.

Within a related aspect of the present invention, vaccines, or immunogenic
5 compositions, for prophylactic or therapeutic use are provided. Such vaccines comprise a polypeptide or polynucleotide as described above and an immunostimulant.

The present invention further provides pharmaceutical compositions that comprise: (a) an antibody or antigen-binding fragment thereof that specifically binds to a lung tumor protein; and (b) a physiologically acceptable carrier.

10 Within further aspects, the present invention provides pharmaceutical compositions comprising: (a) an antigen presenting cell that expresses a polypeptide as described above and (b) a pharmaceutically acceptable carrier or excipient. Antigen presenting cells include dendritic cells, macrophages, monocytes, fibroblasts and B cells.

Within related aspects, vaccines, or immunogenic compositions, are
15 provided that comprise: (a) an antigen presenting cell that expresses a polypeptide as described above and (b) an immunostimulant.

The present invention further provides, in other aspects, fusion proteins that comprise at least one polypeptide as described above, as well as polynucleotides encoding such fusion proteins.

20 Within related aspects, pharmaceutical compositions comprising a fusion protein, or a polynucleotide encoding a fusion protein, in combination with a physiologically acceptable carrier are provided.

Vaccines, or immunogenic compositions, are further provided, within other aspects, that comprise a fusion protein, or a polynucleotide encoding a fusion protein, in
25 combination with an immunostimulant.

Within further aspects, the present invention provides methods for inhibiting the development of a cancer in a patient, comprising administering to a patient a pharmaceutical composition or immunogenic composition as recited above. The patient

may be afflicted with lung cancer, in which case the methods provide treatment for the disease, or patient considered at risk for such a disease may be treated prophylactically.

The present invention further provides, within other aspects, methods for removing tumor cells from a biological sample, comprising contacting a biological sample
5 with T cells that specifically react with a lung tumor protein, wherein the step of contacting is performed under conditions and for a time sufficient to permit the removal of cells expressing the protein from the sample.

Within related aspects, methods are provided for inhibiting the development of a cancer in a patient, comprising administering to a patient a biological sample treated as
10 described above.

Methods are further provided, within other aspects, for stimulating and/or expanding T cells specific for a lung tumor protein, comprising contacting T cells with one or more of: (i) a polypeptide as described above; (ii) a polynucleotide encoding such a polypeptide; and/or (iii) an antigen presenting cell that expresses such a polypeptide; under
15 conditions and for a time sufficient to permit the stimulation and/or expansion of T cells. Isolated T cell populations comprising T cells prepared as described above are also provided.

Within further aspects, the present invention provides methods for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective
20 amount of a T cell population as described above.

The present invention further provides methods for inhibiting the development of a cancer in a patient, comprising the steps of: (a) incubating CD4⁺ and/or CD8⁺ T cells isolated from a patient with one or more of: (i) a polypeptide comprising at least an immunogenic portion of a lung tumor protein; (ii) a polynucleotide encoding such
25 a polypeptide; and (iii) an antigen-presenting cell that expressed such a polypeptide; and (b) administering to the patient an effective amount of the proliferated T cells, and thereby inhibiting the development of a cancer in the patient. Proliferated cells may, but need not, be cloned prior to administration to the patient.

Within further aspects, the present invention provides methods for determining the presence or absence of a cancer in a patient, comprising: (a) contacting a biological sample obtained from a patient with a binding agent that binds to a polypeptide as recited above; (b) detecting in the sample an amount of polypeptide that binds to the binding agent; and (c) comparing the amount of polypeptide with a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient. Within preferred embodiments, the binding agent is an antibody, more preferably a monoclonal antibody. The cancer may be lung cancer.

The present invention also provides, within other aspects, methods for monitoring the progression of a cancer in a patient. Such methods comprise the steps of: (a) contacting a biological sample obtained from a patient at a first point in time with a binding agent that binds to a polypeptide as recited above; (b) detecting in the sample an amount of polypeptide that binds to the binding agent; (c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and (d) comparing the amount of polypeptide detected in step (c) with the amount detected in step (b) and therefrom monitoring the progression of the cancer in the patient.

The present invention further provides, within other aspects, methods for determining the presence or absence of a cancer in a patient, comprising the steps of: (a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes a lung tumor protein; (b) detecting in the sample a level of a polynucleotide, preferably mRNA, that hybridizes to the oligonucleotide; and (c) comparing the level of polynucleotide that hybridizes to the oligonucleotide with a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient. Within certain embodiments, the amount of mRNA is detected via polymerase chain reaction using, for example, at least one oligonucleotide primer that hybridizes to a polynucleotide encoding a polypeptide as recited above, or a complement of such a polynucleotide. Within other embodiments, the amount of mRNA is detected using a hybridization technique, employing an

oligonucleotide probe that hybridizes to a polynucleotide that encodes a polypeptide as recited above, or a complement of such a polynucleotide.

In related aspects, methods are provided for monitoring the progression of a cancer in a patient, comprising the steps of: (a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes a lung tumor protein; (b) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide; (c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and (d) comparing the amount of polynucleotide detected in step (c) with the amount detected in step (b) and therefrom monitoring the progression of the cancer in the patient.

Within further aspects, the present invention provides antibodies, such as monoclonal antibodies, that bind to a polypeptide as described above, as well as diagnostic kits comprising such antibodies. Diagnostic kits comprising one or more oligonucleotide probes or primers as described above are also provided.

These and other aspects of the present invention will become apparent upon reference to the following detailed description. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

SEQUENCE IDENTIFIERS

SEQ ID NO: 1 is the determined cDNA sequence for clone #19038, also referred to as L845P.

SEQ ID NO: 2 is the determined cDNA sequence for clone #19036.

SEQ ID NO: 3 is the determined cDNA sequence for clone #19034.

SEQ ID NO: 4 is the determined cDNA sequence for clone #19033.

SEQ ID NO: 5 is the determined cDNA sequence for clone #19032.

SEQ ID NO: 6 is the determined cDNA sequence for clone #19030, also referred to as L559S.

SEQ ID NO: 7 is the determined cDNA sequence for clone #19029.

SEQ ID NO: 8 is the determined cDNA sequence for clone #19025.

SEQ ID NO: 9 is the determined cDNA sequence for clone #19023.

SEQ ID NO: 10 is the determined cDNA sequence for clone #18929.

SEQ ID NO: 11 is the determined cDNA sequence for clone #19010.

SEQ ID NO: 12 is the determined cDNA sequence for clone #19009.

5 SEQ ID NO: 13 is the determined cDNA sequence for clones #19005, 19007, 19016 and 19017.

SEQ ID NO: 14 is the determined cDNA sequence for clone #19004.

SEQ ID NO: 15 is the determined cDNA sequence for clones #19002 and 18965.

10 SEQ ID NO: 16 is the determined cDNA sequence for clone #18998.

SEQ ID NO: 17 is the determined cDNA sequence for clone #18997.

SEQ ID NO: 18 is the determined cDNA sequence for clone #18996.

SEQ ID NO: 19 is the determined cDNA sequence for clone #18995.

15 SEQ ID NO: 20 is the determined cDNA sequence for clone #18994, also known as L846P.

SEQ ID NO: 21 is the determined cDNA sequence for clone #18992.

SEQ ID NO: 22 is the determined cDNA sequence for clone #18991.

SEQ ID NO: 23 is the determined cDNA sequence for clone #18990, also referred to as clone #20111.

20 SEQ ID NO: 24 is the determined cDNA sequence for clone #18987.

SEQ ID NO: 25 is the determined cDNA sequence for clone #18985, also referred as L839P.

SEQ ID NO: 26 is the determined cDNA sequence for clone #18984, also referred to as L847P.

25 SEQ ID NO: 27 is the determined cDNA sequence for clone #18983.

SEQ ID NO: 28 is the determined cDNA sequence for clones #18976 and 18980.

SEQ ID NO: 29 is the determined cDNA sequence for clone #18975.

SEQ ID NO: 30 is the determined cDNA sequence for clone #18974.

SEQ ID NO: 31 is the determined cDNA sequence for clone #18973.

SEQ ID NO: 32 is the determined cDNA sequence for clone #18972.

SEQ ID NO: 33 is the determined cDNA sequence for clone #18971, also referred to as L801P.

5 SEQ ID NO: 34 is the determined cDNA sequence for clone #18970.

SEQ ID NO: 35 is the determined cDNA sequence for clone #18966.

SEQ ID NO: 36 is the determined cDNA sequence for clones #18964, 18968 and 19039.

SEQ ID NO: 37 is the determined cDNA sequence for clone #18960.

10 SEQ ID NO: 38 is the determined cDNA sequence for clone #18959.

SEQ ID NO: 39 is the determined cDNA sequence for clones #18958 and 18982.

SEQ ID NO: 40 is the determined cDNA sequence for clones #18956 and 19015.

15 SEQ ID NO: 41 is the determined cDNA sequence for clone #18954, also referred to L848P.

SEQ ID NO: 42 is the determined cDNA sequence for clone #18951.

SEQ ID NO: 43 is the determined cDNA sequence for clone #18950.

20 SEQ ID NO: 44 is the determined cDNA sequence for clones #18949 and 19024, also referred to as L844P.

SEQ ID NO: 45 is the determined cDNA sequence for clone #18948.

SEQ ID NO: 46 is the determined cDNA sequence for clone #18947, also referred to as L840P.

25 SEQ ID NO: 47 is the determined cDNA sequence for clones #18946, 18953, 18969 and 19027.

SEQ ID NO: 48 is the determined cDNA sequence for clone #18942.

SEQ ID NO: 49 is the determined cDNA sequence for clone #18940, 18962, 18963, 19006, 19008, 19000, and 19031.

SEQ ID NO: 50 is the determined cDNA sequence for clone #18939.

SEQ ID NO: 51 is the determined cDNA sequence for clones #18938 and 18952.

SEQ ID NO: 52 is the determined cDNA sequence for clone #18938.

SEQ ID NO: 53 is the determined cDNA sequence for clone #18937.

5 SEQ ID NO: 54 is the determined cDNA sequence for clones #18934, 18935, 18993 and 19022, also referred to as L548S.

SEQ ID NO: 55 is the determined cDNA sequence for clone #18932.

SEQ ID NO: 56 is the determined cDNA sequence for clones #18931 and 18936.

10 SEQ ID NO: 57 is the determined cDNA sequence for clone #18930.

SEQ ID NO: 58 is the determined cDNA sequence for clone #19014, also referred to as L773P.

SEQ ID NO: 59 is the determined cDNA sequence for clone #19127.

15 SEQ ID NO: 60 is the determined cDNA sequence for clones #19057 and 19064.

SEQ ID NO: 61 is the determined cDNA sequence for clone #19122.

SEQ ID NO: 62 is the determined cDNA sequence for clones #19120 and 18121.

SEQ ID NO: 63 is the determined cDNA sequence for clone #19118.

20 SEQ ID NO: 64 is the determined cDNA sequence for clone #19117.

SEQ ID NO: 65 is the determined cDNA sequence for clone #19116.

SEQ ID NO: 66 is the determined cDNA sequence for clone #19114.

SEQ ID NO: 67 is the determined cDNA sequence for clone #19112, also known as L561S.

25 SEQ ID NO: 68 is the determined cDNA sequence for clone #19110.

SEQ ID NO: 69 is the determined cDNA sequence for clone #19107, also referred to as L552S.

SEQ ID NO: 70 is the determined cDNA sequence for clone #19106, also referred to as L547S.

SEQ ID NO: 71 is the determined cDNA sequence for clones #19105 and 19111.

SEQ ID NO: 72 is the determined cDNA sequence for clone #19099.

5 SEQ ID NO: 73 is the determined cDNA sequence for clones #19095, 19104 and 19125, also referred to as L549S.

SEQ ID NO: 74 is the determined cDNA sequence for clone #19094.

SEQ ID NO: 75 is the determined cDNA sequence for clones #19089 and 19101.

SEQ ID NO: 76 is the determined cDNA sequence for clone #19088.

10 SEQ ID NO: 77 is the determined cDNA sequence for clones #19087, 19092, 19096, 19100 and 19119.

SEQ ID NO: 78 is the determined cDNA sequence for clone #19086.

SEQ ID NO: 79 is the determined cDNA sequence for clone #19085, also referred to as L550S.

15 SEQ ID NO: 80 is the determined cDNA sequence for clone #19084, also referred to as clone #19079.

SEQ ID NO: 81 is the determined cDNA sequence for clone #19082.

SEQ ID NO: 82 is the determined cDNA sequence for clone #19080.

SEQ ID NO: 83 is the determined cDNA sequence for clone #19077.

20 SEQ ID NO: 84 is the determined cDNA sequence for clone #19076, also referred to as L551S.

SEQ ID NO: 85 is the determined cDNA sequence for clone #19074, also referred to as clone #20102.

25 SEQ ID NO: 86 is the determined cDNA sequence for clone #19073, also referred to as L560S.

SEQ ID NO: 87 is the determined cDNA sequence for clones #19072 and 19115.

SEQ ID NO: 88 is the determined cDNA sequence for clone #19071.

SEQ ID NO: 89 is the determined cDNA sequence for clone #19070.

SEQ ID NO: 90 is the determined cDNA sequence for clone #19069.

SEQ ID NO: 91 is the determined cDNA sequence for clone #19068, also referred to L563S.

SEQ ID NO: 92 is the determined cDNA sequence for clone #19066.

5 SEQ ID NO: 93 is the determined cDNA sequence for clone #19065.

SEQ ID NO: 94 is the determined cDNA sequence for clone #19063.

SEQ ID NO: 95 is the determined cDNA sequence for clones #19061, 19081, 19108 and 19109.

10 SEQ ID NO: 96 is the determined cDNA sequence for clones #19060, 19067 and 19083, also referred to as L548S.

SEQ ID NO: 97 is the determined cDNA sequence for clones #19059 and 19062.

SEQ ID NO: 98 is the determined cDNA sequence for clone #19058.

SEQ ID NO: 99 is the determined cDNA sequence for clone #19124.

15 SEQ ID NO: 100 is the determined cDNA sequence for clone #18929.

SEQ ID NO: 101 is the determined cDNA sequence for clone #18422.

SEQ ID NO: 102 is the determined cDNA sequence for clone #18425.

SEQ ID NO: 103 is the determined cDNA sequence for clone #18431.

SEQ ID NO: 104 is the determined cDNA sequence for clone #18433.

20 SEQ ID NO: 105 is the determined cDNA sequence for clone #18444.

SEQ ID NO: 106 is the determined cDNA sequence for clone #18449.

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SEQ ID NO: 108 is the determined cDNA sequence for clone #18452.

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25 SEQ ID NO: 110 is the determined cDNA sequence for clone #18457.

SEQ ID NO: 111 is the determined cDNA sequence for clone #18466.

SEQ ID NO: 112 is the determined cDNA sequence for clone #18468.

SEQ ID NO: 113 is the determined cDNA sequence for clone #18471.

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5 SEQ ID NO: 115 is the determined cDNA sequence for clone #18476.
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SEQ ID NO: 131 is the determined cDNA sequence for clone #20670.
SEQ ID NO: 132 is the determined cDNA sequence for clone #20671.
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SEQ ID NO: 135 is the determined cDNA sequence for clone #20679.
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5 SEQ ID NO: 172 is the determined cDNA sequence for clone #19159.

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SEQ ID NO: 175 is a second determined cDNA sequence for clone #19161.2.

10 SEQ ID NO: 176 is the determined cDNA sequence for clone #19162.1.

SEQ ID NO: 177 is the determined cDNA sequence for clone #19166.

SEQ ID NO: 178 is the determined cDNA sequence for clone #19169.

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SEQ ID NO: 180 is a first determined cDNA sequence for clone #19173.1.

15 SEQ ID NO: 181 is a second determined cDNA sequence for clone #19173.2.

SEQ ID NO: 182 is the determined cDNA sequence for clone #19174.1.

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20 SEQ ID NO: 185 is the determined cDNA sequence for clone #19178.

SEQ ID NO: 186 is the determined cDNA sequence for clone #19179.1.

SEQ ID NO: 187 is the determined cDNA sequence for clone #19179.2.

SEQ ID NO: 188 is the determined cDNA sequence for clone #19180.

SEQ ID NO: 189 is a first determined cDNA sequence for clone #19182.1.

25 SEQ ID NO: 190 is a second determined cDNA sequence for clone #19182.2.

SEQ ID NO: 191 is the determined cDNA sequence for clone #19183.1.

SEQ ID NO: 192 is the determined cDNA sequence for clone #19185.1.

SEQ ID NO: 193 is the determined cDNA sequence for clone #19187.

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 SEQ ID NO: 208 is a first determined cDNA sequence for clone #19206.1.
 SEQ ID NO: 209 is a second determined cDNA sequence for clone
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 SEQ ID NO: 211 is the determined cDNA sequence for clone #19208.
 SEQ ID NO: 212 is a first determined cDNA sequence for clone #19211.1.
 SEQ ID NO: 213 is a second determined cDNA sequence for clone
 25 #19211.2.
 SEQ ID NO: 214 is a first determined cDNA sequence for clone #19214.1.
 SEQ ID NO: 215 is a second determined cDNA sequence for clone
 #19214.2.
 SEQ ID NO: 216 is the determined cDNA sequence for clone #19215.

SEQ ID NO: 217 is a first determined cDNA sequence for clone #19217. 2.

SEQ ID NO: 218 is a second determined cDNA sequence for clone
#19217.2.

SEQ ID NO: 219 is a first determined cDNA sequence for clone #19218.1.

5 SEQ ID NO: 220 is a second determined cDNA sequence for clone
#19218.2.

SEQ ID NO: 221 is a first determined cDNA sequence for clone #19220.1.

SEQ ID NO: 222 is a second determined cDNA sequence for clone
#19220.2.

10 SEQ ID NO: 223 is the determined cDNA sequence for clone #22015.

SEQ ID NO: 224 is the determined cDNA sequence for clone #22017.

SEQ ID NO: 225 is the determined cDNA sequence for clone #22019.

SEQ ID NO: 226 is the determined cDNA sequence for clone #22020.

SEQ ID NO: 227 is the determined cDNA sequence for clone #22023.

15 SEQ ID NO: 228 is the determined cDNA sequence for clone #22026.

SEQ ID NO: 229 is the determined cDNA sequence for clone #22027.

SEQ ID NO: 230 is the determined cDNA sequence for clone #22028.

SEQ ID NO: 231 is the determined cDNA sequence for clone #22032.

SEQ ID NO: 232 is the determined cDNA sequence for clone #22037.

20 SEQ ID NO: 233 is the determined cDNA sequence for clone #22045.

SEQ ID NO: 234 is the determined cDNA sequence for clone #22048.

SEQ ID NO: 235 is the determined cDNA sequence for clone #22050.

SEQ ID NO: 236 is the determined cDNA sequence for clone #22052.

SEQ ID NO: 237 is the determined cDNA sequence for clone #22053.

25 SEQ ID NO: 238 is the determined cDNA sequence for clone #22057.

SEQ ID NO: 239 is the determined cDNA sequence for clone #22066.

SEQ ID NO: 240 is the determined cDNA sequence for clone #22077.

SEQ ID NO: 241 is the determined cDNA sequence for clone #22085.

SEQ ID NO: 242 is the determined cDNA sequence for clone #22105.

5 SEQ ID NO: 243 is the determined cDNA sequence for clone #22108.
SEQ ID NO: 244 is the determined cDNA sequence for clone #22109.
SEQ ID NO: 245 is the determined cDNA sequence for clone #24842.
SEQ ID NO: 246 is the determined cDNA sequence for clone #24843.
SEQ ID NO: 247 is the determined cDNA sequence for clone #24845.
SEQ ID NO: 248 is the determined cDNA sequence for clone #24851.
SEQ ID NO: 249 is the determined cDNA sequence for clone #24852.
SEQ ID NO: 250 is the determined cDNA sequence for clone #24853.
SEQ ID NO: 251 is the determined cDNA sequence for clone #24854.
10 SEQ ID NO: 252 is the determined cDNA sequence for clone #24855.
SEQ ID NO: 253 is the determined cDNA sequence for clone #24860.
SEQ ID NO: 254 is the determined cDNA sequence for clone #24864.
SEQ ID NO: 255 is the determined cDNA sequence for clone #24866.
SEQ ID NO: 256 is the determined cDNA sequence for clone #24867.
15 SEQ ID NO: 257 is the determined cDNA sequence for clone #24868.
SEQ ID NO: 258 is the determined cDNA sequence for clone #24869.
SEQ ID NO: 259 is the determined cDNA sequence for clone #24870.
SEQ ID NO: 260 is the determined cDNA sequence for clone #24872.
SEQ ID NO: 261 is the determined cDNA sequence for clone #24873.
20 SEQ ID NO: 262 is the determined cDNA sequence for clone #24875.
SEQ ID NO: 263 is the determined cDNA sequence for clone #24882.
SEQ ID NO: 264 is the determined cDNA sequence for clone #24885.
SEQ ID NO: 265 is the determined cDNA sequence for clone #24886.
SEQ ID NO: 266 is the determined cDNA sequence for clone #24887.
25 SEQ ID NO: 267 is the determined cDNA sequence for clone #24888.
SEQ ID NO: 268 is the determined cDNA sequence for clone #24890.
SEQ ID NO: 269 is the determined cDNA sequence for clone #24896.
SEQ ID NO: 270 is the determined cDNA sequence for clone #24897.
SEQ ID NO: 271 is the determined cDNA sequence for clone #24899.

5 SEQ ID NO: 272 is the determined cDNA sequence for clone #24901.
SEQ ID NO: 273 is the determined cDNA sequence for clone #24902.
SEQ ID NO: 274 is the determined cDNA sequence for clone #24906.
SEQ ID NO: 275 is the determined cDNA sequence for clone #24912.
SEQ ID NO: 276 is the determined cDNA sequence for clone #24913.
SEQ ID NO: 277 is the determined cDNA sequence for clone #24920.
SEQ ID NO: 278 is the determined cDNA sequence for clone #24927.
SEQ ID NO: 279 is the determined cDNA sequence for clone #24930.
10 SEQ ID NO: 280 is the determined cDNA sequence for clone #26938.
SEQ ID NO: 281 is the determined cDNA sequence for clone #26939.
SEQ ID NO: 282 is the determined cDNA sequence for clone #26943.
SEQ ID NO: 283 is the determined cDNA sequence for clone #26948.
SEQ ID NO: 284 is the determined cDNA sequence for clone #26951.
SEQ ID NO: 285 is the determined cDNA sequence for clone #26955.
15 SEQ ID NO: 286 is the determined cDNA sequence for clone #26956.
SEQ ID NO: 287 is the determined cDNA sequence for clone #26959.
SEQ ID NO: 288 is the determined cDNA sequence for clone #26961.
SEQ ID NO: 289 is the determined cDNA sequence for clone #26962.
SEQ ID NO: 290 is the determined cDNA sequence for clone #26964.
20 SEQ ID NO: 291 is the determined cDNA sequence for clone #26966.
SEQ ID NO: 292 is the determined cDNA sequence for clone #26968.
SEQ ID NO: 293 is the determined cDNA sequence for clone #26972.
SEQ ID NO: 294 is the determined cDNA sequence for clone #26973.
SEQ ID NO: 295 is the determined cDNA sequence for clone #26974.
25 SEQ ID NO: 296 is the determined cDNA sequence for clone #26976.
SEQ ID NO: 297 is the determined cDNA sequence for clone #26977.
SEQ ID NO: 298 is the determined cDNA sequence for clone #26979.
SEQ ID NO: 299 is the determined cDNA sequence for clone #26980.
SEQ ID NO: 300 is the determined cDNA sequence for clone #26981.

- 006280 "E9T5960
- 5 SEQ ID NO: 301 is the determined cDNA sequence for clone #26984.
SEQ ID NO: 302 is the determined cDNA sequence for clone #26985.
SEQ ID NO: 303 is the determined cDNA sequence for clone #26986.
SEQ ID NO: 304 is the determined cDNA sequence for clone #26993.
SEQ ID NO: 305 is the determined cDNA sequence for clone #26994.
SEQ ID NO: 306 is the determined cDNA sequence for clone #26995.
SEQ ID NO: 307 is the determined cDNA sequence for clone #27003.
SEQ ID NO: 308 is the determined cDNA sequence for clone #27005.
SEQ ID NO: 309 is the determined cDNA sequence for clone #27010.
10 SEQ ID NO: 310 is the determined cDNA sequence for clone #27011.
SEQ ID NO: 311 is the determined cDNA sequence for clone #27013.
SEQ ID NO: 312 is the determined cDNA sequence for clone #27016
SEQ ID NO: 313 is the determined cDNA sequence for clone #27017.
SEQ ID NO: 314 is the determined cDNA sequence for clone #27019.
15 SEQ ID NO: 315 is the determined cDNA sequence for clone #27028.
SEQ ID NO: 316 is the full-length cDNA sequence for clone #19060.
SEQ ID NO: 317 is the full-length cDNA sequence for clone #18964.
SEQ ID NO: 318 is the full-length cDNA sequence for clone #18929.
SEQ ID NO: 319 is the full-length cDNA sequence for clone #18991.
20 SEQ ID NO: 320 is the full-length cDNA sequence for clone #18996.
SEQ ID NO: 321 is the full-length cDNA sequence for clone #18966.
SEQ ID NO: 322 is the full-length cDNA sequence for clone #18951.
SEQ ID NO: 323 is the full-length cDNA sequence for clone #18973 (also
known as L516S).
25 SEQ ID NO: 324 is the amino acid sequence for clone #19060.
SEQ ID NO: 325 is the amino acid sequence for clone #19063.
SEQ ID NO: 326 is the amino acid sequence for clone #19077.
SEQ ID NO: 327 is the amino acid sequence for clone #19110.
SEQ ID NO: 328 is the amino acid sequence for clone #19122.

SEQ ID NO: 329 is the amino acid sequence for clone #19118.

SEQ ID NO: 330 is the amino acid sequence for clone #19080.

SEQ ID NO: 331 is the amino acid sequence for clone #19127.

SEQ ID NO: 332 is the amino acid sequence for clone #19117.

5 SEQ ID NO: 333 is the amino acid sequence for clone #19095, also referred
to L549S.

SEQ ID NO: 334 is the amino acid sequence for clone #18964.

SEQ ID NO: 335 is the amino acid sequence for clone #18929.

SEQ ID NO: 336 is the amino acid sequence for clone #18991.

10 SEQ ID NO: 337 is the amino acid sequence for clone #18996.

SEQ ID NO: 338 is the amino acid sequence for clone #18966.

SEQ ID NO: 339 is the amino acid sequence for clone #18951.

SEQ ID NO: 340 is the amino acid sequence for clone #18973.

SEQ ID NO: 341 is the determined cDNA sequence for clone 26461.

15 SEQ ID NO: 342 is the determined cDNA sequence for clone 26462.

SEQ ID NO: 343 is the determined cDNA sequence for clone 26463.

SEQ ID NO: 344 is the determined cDNA sequence for clone 26464.

SEQ ID NO: 345 is the determined cDNA sequence for clone 26465.

SEQ ID NO: 346 is the determined cDNA sequence for clone 26466.

20 SEQ ID NO: 347 is the determined cDNA sequence for clone 26467.

SEQ ID NO: 348 is the determined cDNA sequence for clone 26468.

SEQ ID NO: 349 is the determined cDNA sequence for clone 26469.

SEQ ID NO: 350 is the determined cDNA sequence for clone 26470.

SEQ ID NO: 351 is the determined cDNA sequence for clone 26471.

25 SEQ ID NO: 352 is the determined cDNA sequence for clone 26472.

SEQ ID NO: 353 is the determined cDNA sequence for clone 26474.

SEQ ID NO: 354 is the determined cDNA sequence for clone 26475.

SEQ ID NO: 355 is the determined cDNA sequence for clone 26476.

SEQ ID NO: 356 is the determined cDNA sequence for clone 26477.

5 SEQ ID NO: 357 is the determined cDNA sequence for clone 26478.
SEQ ID NO: 358 is the determined cDNA sequence for clone 26479.
SEQ ID NO: 359 is the determined cDNA sequence for clone 26480.
SEQ ID NO: 360 is the determined cDNA sequence for clone 26481.
SEQ ID NO: 361 is the determined cDNA sequence for clone 26482
SEQ ID NO: 362 is the determined cDNA sequence for clone 26483.
SEQ ID NO: 363 is the determined cDNA sequence for clone 26484.
SEQ ID NO: 364 is the determined cDNA sequence for clone 26485.
SEQ ID NO: 365 is the determined cDNA sequence for clone 26486.
10 SEQ ID NO: 366 is the determined cDNA sequence for clone 26487.
SEQ ID NO: 367 is the determined cDNA sequence for clone 26488.
SEQ ID NO: 368 is the determined cDNA sequence for clone 26489.
SEQ ID NO: 369 is the determined cDNA sequence for clone 26490.
SEQ ID NO: 370 is the determined cDNA sequence for clone 26491.
15 SEQ ID NO: 371 is the determined cDNA sequence for clone 26492.
SEQ ID NO: 372 is the determined cDNA sequence for clone 26493.
SEQ ID NO: 373 is the determined cDNA sequence for clone 26494.
SEQ ID NO: 374 is the determined cDNA sequence for clone 26495.
SEQ ID NO: 375 is the determined cDNA sequence for clone 26496.
20 SEQ ID NO: 376 is the determined cDNA sequence for clone 26497.
SEQ ID NO: 377 is the determined cDNA sequence for clone 26498.
SEQ ID NO: 378 is the determined cDNA sequence for clone 26499.
SEQ ID NO: 379 is the determined cDNA sequence for clone 26500.
SEQ ID NO: 380 is the determined cDNA sequence for clone 26501.
25 SEQ ID NO: 381 is the determined cDNA sequence for clone 26502.
SEQ ID NO: 382 is the determined cDNA sequence for clone 26503.
SEQ ID NO: 383 is the determined cDNA sequence for clone 26504.
SEQ ID NO: 384 is the determined cDNA sequence for clone 26505.
SEQ ID NO: 385 is the determined cDNA sequence for clone 26506.

5 SEQ ID NO: 386 is the determined cDNA sequence for clone 26507.
SEQ ID NO: 387 is the determined cDNA sequence for clone 26508.
SEQ ID NO: 388 is the determined cDNA sequence for clone 26509.
SEQ ID NO: 389 is the determined cDNA sequence for clone 26511.
SEQ ID NO: 390 is the determined cDNA sequence for clone 26513.
SEQ ID NO: 391 is the determined cDNA sequence for clone 26514.
SEQ ID NO: 392 is the determined cDNA sequence for clone 26515.
SEQ ID NO: 393 is the determined cDNA sequence for clone 26516.
SEQ ID NO: 394 is the determined cDNA sequence for clone 26517.
10 SEQ ID NO: 395 is the determined cDNA sequence for clone 26518.
SEQ ID NO: 396 is the determined cDNA sequence for clone 26519.
SEQ ID NO: 397 is the determined cDNA sequence for clone 26520.
SEQ ID NO: 398 is the determined cDNA sequence for clone 26521.
SEQ ID NO: 399 is the determined cDNA sequence for clone 26522.
15 SEQ ID NO: 400 is the determined cDNA sequence for clone 26523.
SEQ ID NO: 401 is the determined cDNA sequence for clone 26524.
SEQ ID NO: 402 is the determined cDNA sequence for clone 26526.
SEQ ID NO: 403 is the determined cDNA sequence for clone 26527.
SEQ ID NO: 404 is the determined cDNA sequence for clone 26528.
20 SEQ ID NO: 405 is the determined cDNA sequence for clone 26529.
SEQ ID NO: 406 is the determined cDNA sequence for clone 26530.
SEQ ID NO: 407 is the determined cDNA sequence for clone 26532.
SEQ ID NO: 408 is the determined cDNA sequence for clone 26533.
SEQ ID NO: 409 is the determined cDNA sequence for clone 26534.
25 SEQ ID NO: 410 is the determined cDNA sequence for clone 26535.
SEQ ID NO: 411 is the determined cDNA sequence for clone 26536.
SEQ ID NO: 412 is the determined cDNA sequence for clone 26537.
SEQ ID NO: 413 is the determined cDNA sequence for clone 26538.
SEQ ID NO: 414 is the determined cDNA sequence for clone 26540.

5 SEQ ID NO: 415 is the determined cDNA sequence for clone 26541.
SEQ ID NO: 416 is the determined cDNA sequence for clone 26542.
SEQ ID NO: 417 is the determined cDNA sequence for clone 26543.
SEQ ID NO: 418 is the determined cDNA sequence for clone 26544.
SEQ ID NO: 419 is the determined cDNA sequence for clone 26546.
SEQ ID NO: 420 is the determined cDNA sequence for clone 26547.
SEQ ID NO: 421 is the determined cDNA sequence for clone 26548.
SEQ ID NO: 422 is the determined cDNA sequence for clone 26549.
SEQ ID NO: 423 is the determined cDNA sequence for clone 26550.
10 SEQ ID NO: 424 is the determined cDNA sequence for clone 26551.
SEQ ID NO: 425 is the determined cDNA sequence for clone 26552.
SEQ ID NO: 426 is the determined cDNA sequence for clone 26553.
SEQ ID NO: 427 is the determined cDNA sequence for clone 26554.
SEQ ID NO: 428 is the determined cDNA sequence for clone 26556.
15 SEQ ID NO: 429 is the determined cDNA sequence for clone 26557.
SEQ ID NO: 430 is the determined cDNA sequence for clone 27631.
SEQ ID NO: 431 is the determined cDNA sequence for clone 27632.
SEQ ID NO: 432 is the determined cDNA sequence for clone 27633.
SEQ ID NO: 433 is the determined cDNA sequence for clone 27635.
20 SEQ ID NO: 434 is the determined cDNA sequence for clone 27636.
SEQ ID NO: 435 is the determined cDNA sequence for clone 27637.
SEQ ID NO: 436 is the determined cDNA sequence for clone 27638.
SEQ ID NO: 437 is the determined cDNA sequence for clone 27639.
SEQ ID NO: 438 is the determined cDNA sequence for clone 27640.
25 SEQ ID NO: 439 is the determined cDNA sequence for clone 27641.
SEQ ID NO: 440 is the determined cDNA sequence for clone 27642.
SEQ ID NO: 441 is the determined cDNA sequence for clone 27644.
SEQ ID NO: 442 is the determined cDNA sequence for clone 27646.
SEQ ID NO: 443 is the determined cDNA sequence for clone 27647.

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20

25

SEQ ID NO: 450 is the determined cDNA sequence for clone 27657.

SEQ ID NO: 452 is the determined cDNA sequence for clone 27665.

SEQ ID NO: 454 is the determined cDNA sequence for clone 27668.

SEQ ID NO: 456 is the determined cDNA sequence for clone 27671.

SEQ ID NO: 458 is the determined cDNA sequence for clone 27674.

SEQ ID NO: 460 is the determined cDNA sequence for clone 27681.

SEQ ID NO: 462 is the determined cDNA sequence for clone 27683.

SEQ ID NO: 464 is the determined cDNA sequence for clone 27688.

SEQ ID NO: 466 is the determined cDNA sequence for clone 27690.

SEQ ID NO: 468 is the determined cDNA sequence for clone 27699.

SEQ ID NO: 470 is the determined cDNA sequence for clone 27702.

SEQ ID NO: 471 is the determined cDNA sequence for clone 27705.

SEQ ID NO: 472 is the determined cDNA sequence for clone 27706.

5 SEQ ID NO: 473 is the determined cDNA sequence for clone 27707.
SEQ ID NO: 474 is the determined cDNA sequence for clone 27708.
SEQ ID NO: 475 is the determined cDNA sequence for clone 27709.
SEQ ID NO: 476 is the determined cDNA sequence for clone 27710.
SEQ ID NO: 477 is the determined cDNA sequence for clone 27711.
SEQ ID NO: 478 is the determined cDNA sequence for clone 27712.
SEQ ID NO: 479 is the determined cDNA sequence for clone 27713.
SEQ ID NO: 480 is the determined cDNA sequence for clone 27714.
SEQ ID NO: 481 is the determined cDNA sequence for clone 27715.
10 SEQ ID NO: 482 is the determined cDNA sequence for clone 27716.
SEQ ID NO: 483 is the determined cDNA sequence for clone 27717.
SEQ ID NO: 484 is the determined cDNA sequence for clone 27718.
SEQ ID NO: 485 is the determined cDNA sequence for clone 27719.
SEQ ID NO: 486 is the determined cDNA sequence for clone 27720.
15 SEQ ID NO: 487 is the determined cDNA sequence for clone 27722.
SEQ ID NO: 488 is the determined cDNA sequence for clone 27723.
SEQ ID NO: 489 is the determined cDNA sequence for clone 27724.
SEQ ID NO: 490 is the determined cDNA sequence for clone 27726.
SEQ ID NO: 491 is the determined cDNA sequence for clone 25015.
20 SEQ ID NO: 492 is the determined cDNA sequence for clone 25016.
SEQ ID NO: 493 is the determined cDNA sequence for clone 25017.
SEQ ID NO: 494 is the determined cDNA sequence for clone 25018
SEQ ID NO: 495 is the determined cDNA sequence for clone 25030.
SEQ ID NO: 496 is the determined cDNA sequence for clone 25033.
25 SEQ ID NO: 497 is the determined cDNA sequence for clone 25034.
SEQ ID NO: 498 is the determined cDNA sequence for clone 25035.
SEQ ID NO: 499 is the determined cDNA sequence for clone 25036.
SEQ ID NO: 500 is the determined cDNA sequence for clone 25037.
SEQ ID NO: 501 is the determined cDNA sequence for clone 25038.

5 SEQ ID NO: 502 is the determined cDNA sequence for clone 25039.
SEQ ID NO: 503 is the determined cDNA sequence for clone 25040.
SEQ ID NO: 504 is the determined cDNA sequence for clone 25042.
SEQ ID NO: 505 is the determined cDNA sequence for clone 25043.
SEQ ID NO: 506 is the determined cDNA sequence for clone 25044.
SEQ ID NO: 507 is the determined cDNA sequence for clone 25045.
SEQ ID NO: 508 is the determined cDNA sequence for clone 25047.
SEQ ID NO: 509 is the determined cDNA sequence for clone 25048.
10 SEQ ID NO: 510 is the determined cDNA sequence for clone 25049.
SEQ ID NO: 511 is the determined cDNA sequence for clone 25185.
SEQ ID NO: 512 is the determined cDNA sequence for clone 25186.
SEQ ID NO: 513 is the determined cDNA sequence for clone 25187.
SEQ ID NO: 514 is the determined cDNA sequence for clone 25188.
SEQ ID NO: 515 is the determined cDNA sequence for clone 25189.
15 SEQ ID NO: 516 is the determined cDNA sequence for clone 25190.
SEQ ID NO: 517 is the determined cDNA sequence for clone 25193.
SEQ ID NO: 518 is the determined cDNA sequence for clone 25194.
SEQ ID NO: 519 is the determined cDNA sequence for clone 25196.
SEQ ID NO: 520 is the determined cDNA sequence for clone 25198.
20 SEQ ID NO: 521 is the determined cDNA sequence for clone 25199.
SEQ ID NO: 522 is the determined cDNA sequence for clone 25200.
SEQ ID NO: 523 is the determined cDNA sequence for clone 25202.
SEQ ID NO: 524 is the determined cDNA sequence for clone 25364.
SEQ ID NO: 525 is the determined cDNA sequence for clone 25366.
25 SEQ ID NO: 526 is the determined cDNA sequence for clone 25367.
SEQ ID NO: 527 is the determined cDNA sequence for clone 25368.
SEQ ID NO: 528 is the determined cDNA sequence for clone 25369.
SEQ ID NO: 529 is the determined cDNA sequence for clone 25370.
SEQ ID NO: 530 is the determined cDNA sequence for clone 25371.

5 SEQ ID NO: 531 is the determined cDNA sequence for clone 25372.
SEQ ID NO: 532 is the determined cDNA sequence for clone 25373.
SEQ ID NO: 533 is the determined cDNA sequence for clone 25374.
SEQ ID NO: 534 is the determined cDNA sequence for clone 25376.
SEQ ID NO: 535 is the determined cDNA sequence for clone 25377.
SEQ ID NO: 536 is the determined cDNA sequence for clone 25378.
SEQ ID NO: 537 is the determined cDNA sequence for clone 25379.
SEQ ID NO: 538 is the determined cDNA sequence for clone 25380.
SEQ ID NO: 539 is the determined cDNA sequence for clone 25381.
10 SEQ ID NO: 540 is the determined cDNA sequence for clone 25382.
SEQ ID NO: 541 is the determined cDNA sequence for clone 25383.
SEQ ID NO: 542 is the determined cDNA sequence for clone 25385.
SEQ ID NO: 543 is the determined cDNA sequence for clone 25386.
SEQ ID NO: 544 is the determined cDNA sequence for clone 25387.
15 SEQ ID NO: 545 is the determined cDNA sequence for clone 26013.
SEQ ID NO: 546 is the determined cDNA sequence for clone 26014.
SEQ ID NO: 547 is the determined cDNA sequence for clone 26016.
SEQ ID NO: 548 is the determined cDNA sequence for clone 26017.
SEQ ID NO: 549 is the determined cDNA sequence for clone 26018.
20 SEQ ID NO: 550 is the determined cDNA sequence for clone 26019.
SEQ ID NO: 551 is the determined cDNA sequence for clone 26020.
SEQ ID NO: 552 is the determined cDNA sequence for clone 26021.
SEQ ID NO: 553 is the determined cDNA sequence for clone 26022.
SEQ ID NO: 554 is the determined cDNA sequence for clone 26027.
25 SEQ ID NO: 555 is the determined cDNA sequence for clone 26197.
SEQ ID NO: 556 is the determined cDNA sequence for clone 26199.
SEQ ID NO: 557 is the determined cDNA sequence for clone 26201.
SEQ ID NO: 558 is the determined cDNA sequence for clone 26202.
SEQ ID NO: 559 is the determined cDNA sequence for clone 26203.

5 SEQ ID NO: 560 is the determined cDNA sequence for clone 26204.
SEQ ID NO: 561 is the determined cDNA sequence for clone 26205.
SEQ ID NO: 562 is the determined cDNA sequence for clone 26206.
SEQ ID NO: 563 is the determined cDNA sequence for clone 26208.
SEQ ID NO: 564 is the determined cDNA sequence for clone 26211.
SEQ ID NO: 565 is the determined cDNA sequence for clone 26212.
SEQ ID NO: 566 is the determined cDNA sequence for clone 26213.
SEQ ID NO: 567 is the determined cDNA sequence for clone 26214.
SEQ ID NO: 568 is the determined cDNA sequence for clone 26215.
10 SEQ ID NO: 569 is the determined cDNA sequence for clone 26216.
SEQ ID NO: 570 is the determined cDNA sequence for clone 26217.
SEQ ID NO: 571 is the determined cDNA sequence for clone 26218.
SEQ ID NO: 572 is the determined cDNA sequence for clone 26219.
SEQ ID NO: 573 is the determined cDNA sequence for clone 26220.
15 SEQ ID NO: 574 is the determined cDNA sequence for clone 26221.
SEQ ID NO: 575 is the determined cDNA sequence for clone 26224.
SEQ ID NO: 576 is the determined cDNA sequence for clone 26225.
SEQ ID NO: 577 is the determined cDNA sequence for clone 26226.
SEQ ID NO: 578 is the determined cDNA sequence for clone 26227.
20 SEQ ID NO: 579 is the determined cDNA sequence for clone 26228.
SEQ ID NO: 580 is the determined cDNA sequence for clone 26230.
SEQ ID NO: 581 is the determined cDNA sequence for clone 26231.
SEQ ID NO: 582 is the determined cDNA sequence for clone 26234.
SEQ ID NO: 583 is the determined cDNA sequence for clone 26236.
25 SEQ ID NO: 584 is the determined cDNA sequence for clone 26237.
SEQ ID NO: 585 is the determined cDNA sequence for clone 26239.
SEQ ID NO: 586 is the determined cDNA sequence for clone 26240.
SEQ ID NO: 587 is the determined cDNA sequence for clone 26241.
SEQ ID NO: 588 is the determined cDNA sequence for clone 26242.

5 SEQ ID NO: 589 is the determined cDNA sequence for clone 26246.
SEQ ID NO: 590 is the determined cDNA sequence for clone 26247.
SEQ ID NO: 591 is the determined cDNA sequence for clone 26248.
SEQ ID NO: 592 is the determined cDNA sequence for clone 26249.
SEQ ID NO: 593 is the determined cDNA sequence for clone 26250.
SEQ ID NO: 594 is the determined cDNA sequence for clone 26251.
SEQ ID NO: 595 is the determined cDNA sequence for clone 26252.
SEQ ID NO: 596 is the determined cDNA sequence for clone 26253.
SEQ ID NO: 597 is the determined cDNA sequence for clone 26254.
10 SEQ ID NO: 598 is the determined cDNA sequence for clone 26255.
SEQ ID NO: 599 is the determined cDNA sequence for clone 26256.
SEQ ID NO: 600 is the determined cDNA sequence for clone 26257.
SEQ ID NO: 601 is the determined cDNA sequence for clone 26259.
SEQ ID NO: 602 is the determined cDNA sequence for clone 26260.
15 SEQ ID NO: 603 is the determined cDNA sequence for clone 26261.
SEQ ID NO: 604 is the determined cDNA sequence for clone 26262.
SEQ ID NO: 605 is the determined cDNA sequence for clone 26263.
SEQ ID NO: 606 is the determined cDNA sequence for clone 26264.
SEQ ID NO: 607 is the determined cDNA sequence for clone 26265.
20 SEQ ID NO: 608 is the determined cDNA sequence for clone 26266.
SEQ ID NO: 609 is the determined cDNA sequence for clone 26268.
SEQ ID NO: 610 is the determined cDNA sequence for clone 26269.
SEQ ID NO: 611 is the determined cDNA sequence for clone 26271.
SEQ ID NO: 612 is the determined cDNA sequence for clone 26273.
25 SEQ ID NO: 613 is the determined cDNA sequence for clone 26810.
SEQ ID NO: 614 is the determined cDNA sequence for clone 26811.
SEQ ID NO: 615 is the determined cDNA sequence for clone 26812.1.
SEQ ID NO: 616 is the determined cDNA sequence for clone 26812.2.
SEQ ID NO: 617 is the determined cDNA sequence for clone 26813.

5 SEQ ID NO: 618 is the determined cDNA sequence for clone 26814.
SEQ ID NO: 619 is the determined cDNA sequence for clone 26815.
SEQ ID NO: 620 is the determined cDNA sequence for clone 26816.
SEQ ID NO: 621 is the determined cDNA sequence for clone 26818.
SEQ ID NO: 622 is the determined cDNA sequence for clone 26819.
SEQ ID NO: 623 is the determined cDNA sequence for clone 26820.
SEQ ID NO: 624 is the determined cDNA sequence for clone 26821.
SEQ ID NO: 625 is the determined cDNA sequence for clone 26822.
SEQ ID NO: 626 is the determined cDNA sequence for clone 26824.
10 SEQ ID NO: 627 is the determined cDNA sequence for clone 26825.
SEQ ID NO: 628 is the determined cDNA sequence for clone 26826.
SEQ ID NO: 629 is the determined cDNA sequence for clone 26827.
SEQ ID NO: 630 is the determined cDNA sequence for clone 26829.
SEQ ID NO: 631 is the determined cDNA sequence for clone 26830.
15 SEQ ID NO: 632 is the determined cDNA sequence for clone 26831.
SEQ ID NO: 633 is the determined cDNA sequence for clone 26832.
SEQ ID NO: 634 is the determined cDNA sequence for clone 26835.
SEQ ID NO: 635 is the determined cDNA sequence for clone 26836.
SEQ ID NO: 636 is the determined cDNA sequence for clone 26837.
20 SEQ ID NO: 637 is the determined cDNA sequence for clone 26839.
SEQ ID NO: 638 is the determined cDNA sequence for clone 26841.
SEQ ID NO: 639 is the determined cDNA sequence for clone 26843.
SEQ ID NO: 640 is the determined cDNA sequence for clone 26844.
SEQ ID NO: 641 is the determined cDNA sequence for clone 26845.
25 SEQ ID NO: 642 is the determined cDNA sequence for clone 26846.
SEQ ID NO: 643 is the determined cDNA sequence for clone 26847.
SEQ ID NO: 644 is the determined cDNA sequence for clone 26848.
SEQ ID NO: 645 is the determined cDNA sequence for clone 26849.
SEQ ID NO: 646 is the determined cDNA sequence for clone 26850.

5 SEQ ID NO: 647 is the determined cDNA sequence for clone 26851.
SEQ ID NO: 648 is the determined cDNA sequence for clone 26852.
SEQ ID NO: 649 is the determined cDNA sequence for clone 26853.
SEQ ID NO: 650 is the determined cDNA sequence for clone 26854.
SEQ ID NO: 651 is the determined cDNA sequence for clone 26856.
SEQ ID NO: 652 is the determined cDNA sequence for clone 26857.
SEQ ID NO: 653 is the determined cDNA sequence for clone 26858.
SEQ ID NO: 654 is the determined cDNA sequence for clone 26859.
10 SEQ ID NO: 655 is the determined cDNA sequence for clone 26860.
SEQ ID NO: 656 is the determined cDNA sequence for clone 26862.
SEQ ID NO: 657 is the determined cDNA sequence for clone 26863.
SEQ ID NO: 658 is the determined cDNA sequence for clone 26864.
SEQ ID NO: 659 is the determined cDNA sequence for clone 26865.
SEQ ID NO: 660 is the determined cDNA sequence for clone 26867.
15 SEQ ID NO: 661 is the determined cDNA sequence for clone 26868.
SEQ ID NO: 662 is the determined cDNA sequence for clone 26871.
SEQ ID NO: 663 is the determined cDNA sequence for clone 26873.
SEQ ID NO: 664 is the determined cDNA sequence for clone 26875.
SEQ ID NO: 665 is the determined cDNA sequence for clone 26876.
20 SEQ ID NO: 666 is the determined cDNA sequence for clone 26877.
SEQ ID NO: 667 is the determined cDNA sequence for clone 26878.
SEQ ID NO: 668 is the determined cDNA sequence for clone 26880.
SEQ ID NO: 669 is the determined cDNA sequence for clone 26882.
SEQ ID NO: 670 is the determined cDNA sequence for clone 26883.
25 SEQ ID NO: 671 is the determined cDNA sequence for clone 26884.
SEQ ID NO: 672 is the determined cDNA sequence for clone 26885.
SEQ ID NO: 673 is the determined cDNA sequence for clone 26886.
SEQ ID NO: 674 is the determined cDNA sequence for clone 26887.
SEQ ID NO: 675 is the determined cDNA sequence for clone 26888.

SEQ ID NO: 676 is the determined cDNA sequence for clone 26889.
SEQ ID NO: 677 is the determined cDNA sequence for clone 26890.
SEQ ID NO: 678 is the determined cDNA sequence for clone 26892.
SEQ ID NO: 679 is the determined cDNA sequence for clone 26894.
5 SEQ ID NO: 680 is the determined cDNA sequence for clone 26895.
SEQ ID NO: 681 is the determined cDNA sequence for clone 26897.
SEQ ID NO: 682 is the determined cDNA sequence for clone 26898.
SEQ ID NO: 683 is the determined cDNA sequence for clone 26899.
SEQ ID NO: 684 is the determined cDNA sequence for clone 26900.
10 SEQ ID NO: 685 is the determined cDNA sequence for clone 26901.
SEQ ID NO: 686 is the determined cDNA sequence for clone 26903.
SEQ ID NO: 687 is the determined cDNA sequence for clone 26905.
SEQ ID NO: 688 is the determined cDNA sequence for clone 26906.
SEQ ID NO: 689 is the determined cDNA sequence for clone 26708.
15 SEQ ID NO: 690 is the determined cDNA sequence for clone 26709.
SEQ ID NO: 691 is the determined cDNA sequence for clone 26710.
SEQ ID NO: 692 is the determined cDNA sequence for clone 26711.
SEQ ID NO: 693 is the determined cDNA sequence for clone 26712.
SEQ ID NO: 694 is the determined cDNA sequence for clone 26713.
20 SEQ ID NO: 695 is the determined cDNA sequence for clone 26714.
SEQ ID NO: 696 is the determined cDNA sequence for clone 26715.
SEQ ID NO: 697 is the determined cDNA sequence for clone 26716.
SEQ ID NO: 698 is the determined cDNA sequence for clone 26717.
SEQ ID NO: 699 is the determined cDNA sequence for clone 26718.
25 SEQ ID NO: 700 is the determined cDNA sequence for clone 26719.
SEQ ID NO: 701 is the determined cDNA sequence for clone 26720.
SEQ ID NO: 702 is the determined cDNA sequence for clone 26721.
SEQ ID NO: 703 is the determined cDNA sequence for clone 26722.
SEQ ID NO: 704 is the determined cDNA sequence for clone 26723.

5 SEQ ID NO: 705 is the determined cDNA sequence for clone 26724.
SEQ ID NO: 706 is the determined cDNA sequence for clone 26725.
SEQ ID NO: 707 is the determined cDNA sequence for clone 26726.
SEQ ID NO: 708 is the determined cDNA sequence for clone 26727.
SEQ ID NO: 709 is the determined cDNA sequence for clone 26728.
SEQ ID NO: 710 is the determined cDNA sequence for clone 26729.
SEQ ID NO: 711 is the determined cDNA sequence for clone 26730.
SEQ ID NO: 712 is the determined cDNA sequence for clone 26731.
SEQ ID NO: 713 is the determined cDNA sequence for clone 26732.
10 SEQ ID NO: 714 is the determined cDNA sequence for clone 26733.1.
SEQ ID NO: 715 is the determined cDNA sequence for clone 26733.2.
SEQ ID NO: 716 is the determined cDNA sequence for clone 26734.
SEQ ID NO: 717 is the determined cDNA sequence for clone 26735.
SEQ ID NO: 718 is the determined cDNA sequence for clone 26736.
15 SEQ ID NO: 719 is the determined cDNA sequence for clone 26737.
SEQ ID NO: 720 is the determined cDNA sequence for clone 26738.
SEQ ID NO: 721 is the determined cDNA sequence for clone 26739.
SEQ ID NO: 722 is the determined cDNA sequence for clone 26741.
SEQ ID NO: 723 is the determined cDNA sequence for clone 26742.
20 SEQ ID NO: 724 is the determined cDNA sequence for clone 26743.
SEQ ID NO: 725 is the determined cDNA sequence for clone 26744.
SEQ ID NO: 726 is the determined cDNA sequence for clone 26745.
SEQ ID NO: 727 is the determined cDNA sequence for clone 26746.
SEQ ID NO: 728 is the determined cDNA sequence for clone 26747.
25 SEQ ID NO: 729 is the determined cDNA sequence for clone 26748.
SEQ ID NO: 730 is the determined cDNA sequence for clone 26749.
SEQ ID NO: 731 is the determined cDNA sequence for clone 26750.
SEQ ID NO: 732 is the determined cDNA sequence for clone 26751.
SEQ ID NO: 733 is the determined cDNA sequence for clone 26752.

5 SEQ ID NO: 734 is the determined cDNA sequence for clone 26753.
SEQ ID NO: 735 is the determined cDNA sequence for clone 26754.
SEQ ID NO: 736 is the determined cDNA sequence for clone 26755.
SEQ ID NO: 737 is the determined cDNA sequence for clone 26756.
SEQ ID NO: 738 is the determined cDNA sequence for clone 26757.
SEQ ID NO: 739 is the determined cDNA sequence for clone 26758.
SEQ ID NO: 740 is the determined cDNA sequence for clone 26759.
SEQ ID NO: 741 is the determined cDNA sequence for clone 26760.
SEQ ID NO: 742 is the determined cDNA sequence for clone 26761.
10 SEQ ID NO: 743 is the determined cDNA sequence for clone 26762.
SEQ ID NO: 744 is the determined cDNA sequence for clone 26763.
SEQ ID NO: 745 is the determined cDNA sequence for clone 26764.
SEQ ID NO: 746 is the determined cDNA sequence for clone 26765.
SEQ ID NO: 747 is the determined cDNA sequence for clone 26766.
15 SEQ ID NO: 748 is the determined cDNA sequence for clone 26767.
SEQ ID NO: 749 is the determined cDNA sequence for clone 26768.
SEQ ID NO: 750 is the determined cDNA sequence for clone 26769.
SEQ ID NO: 751 is the determined cDNA sequence for clone 26770.
SEQ ID NO: 752 is the determined cDNA sequence for clone 26771.
20 SEQ ID NO: 753 is the determined cDNA sequence for clone 26772.
SEQ ID NO: 754 is the determined cDNA sequence for clone 26773.
SEQ ID NO: 755 is the determined cDNA sequence for clone 26774.
SEQ ID NO: 756 is the determined cDNA sequence for clone 26775.
SEQ ID NO: 757 is the determined cDNA sequence for clone 26776.
25 SEQ ID NO: 758 is the determined cDNA sequence for clone 26777.
SEQ ID NO: 759 is the determined cDNA sequence for clone 26778.
SEQ ID NO: 760 is the determined cDNA sequence for clone 26779.
SEQ ID NO: 761 is the determined cDNA sequence for clone 26781.
SEQ ID NO: 762 is the determined cDNA sequence for clone 26782.

5 SEQ ID NO: 763 is the determined cDNA sequence for clone 26783.
 SEQ ID NO: 764 is the determined cDNA sequence for clone 26784.
 SEQ ID NO: 765 is the determined cDNA sequence for clone 26785.
 SEQ ID NO: 766 is the determined cDNA sequence for clone 26786.
 SEQ ID NO: 767 is the determined cDNA sequence for clone 26787.
 SEQ ID NO: 768 is the determined cDNA sequence for clone 26788.
 SEQ ID NO: 769 is the determined cDNA sequence for clone 26790.
 SEQ ID NO: 770 is the determined cDNA sequence for clone 26791.
 SEQ ID NO: 771 is the determined cDNA sequence for clone 26792.
 10 SEQ ID NO: 772 is the determined cDNA sequence for clone 26793.
 SEQ ID NO: 773 is the determined cDNA sequence for clone 26794.
 SEQ ID NO: 774 is the determined cDNA sequence for clone 26795.
 SEQ ID NO: 775 is the determined cDNA sequence for clone 26796.
 SEQ ID NO: 776 is the determined cDNA sequence for clone 26797.
 15 SEQ ID NO: 777 is the determined cDNA sequence for clone 26798.
 SEQ ID NO: 778 is the determined cDNA sequence for clone 26800.
 SEQ ID NO: 779 is the determined cDNA sequence for clone 26801.
 SEQ ID NO: 780 is the determined cDNA sequence for clone 26802.
 SEQ ID NO: 781 is the determined cDNA sequence for clone 26803.
 20 SEQ ID NO: 782 is the determined cDNA sequence for clone 26804.
 SEQ ID NO: 783 is the amino acid sequence for L773P.
 SEQ ID NO: 784 is the determined DNA sequence of the L773P expression
 construct.
 SEQ ID NO: 785 is the determined DNA sequence of the L773PA
 25 expression construct.
 SEQ ID NO: 786 is a predicted amino acid sequence for L552S.
 SEQ ID NO: 787 is a predicted amino acid sequence for L840P.
 SEQ ID NO: 788 is the full-length cDNA sequence for L548S.
 SEQ ID NO: 789 is the amino acid sequence encoded by SEQ ID NO: 788.

SEQ ID NO: 790 is an extended cDNA sequence for L552S.

SEQ ID NO: 791 is the predicted amino acid sequence encoded by the cDNA sequence of SEQ ID NO: 790.

5 L552S.
SEQ ID NO: 792 is the determined cDNA sequence for an isoform of

NO: 792.
SEQ ID NO: 793 is the predicted amino acid sequence encoded by SEQ ID

10 NO: 794.
SEQ ID NO: 794 is an extended cDNA sequence for L840P.
SEQ ID NO: 795 is the predicted amino acid sequence encoded by SEQ DI

SEQ ID NO: 796 is an extended cDNA sequence for L801P.
SEQ ID NO: 797 is a first predicted amino acid sequence encoded by SEQ
ID NO: 796.

15 SEQ ID NO: 798 is a second predicted amino acid sequence encoded by
SEQ ID NO: 796.
SEQ ID NO: 799 is a third predicted amino acid sequence encoded by SEQ
ID NO: 796.

20 SEQ ID NO: 800 is the determined full-length sequence for L844P.
SEQ ID NO: 801 is the 5' consensus cDNA sequence for L551S.
SEQ ID NO: 802 is the 3' consensus cDNA sequence for L551S.
SEQ ID NO: 803 is the cDNA sequence for STY8.

25 SEQ ID NO: 804 is an extended cDNA sequence for L551S.
SEQ ID NO: 805 is the amino acid sequence for STY8.
SEQ ID NO: 806 is the extended amino acid sequence for L551S.
SEQ ID NO: 807 is the determined full-length cDNA sequence for L773P.
SEQ ID NO: 808 is the full-length cDNA sequence of L552S.
SEQ ID NO: 809 is the full-length amino acid sequence of L552S.
SEQ ID NO: 810 is the determined cDNA sequence of clone 50989.
SEQ ID NO: 811 is the determined cDNA sequence of clone 50990.



Table 1A

SEQ ID NO	CLONE IDENTIFIER	SEQ ID NO	CLONE IDENTIFIER
828	R0126:A02	869	R0126:D12
829	R0126:A03	870	R0126:E01
830	R0126:A05	871	R0126:E02
831	R0126:A06	872	R0126:E03
832	R0126:A08	873	R0126:E04
833	R0126:A09	874	R0126:E05
834	R0126:A10	875	R0126:E06
835	R0126:A11	876	R0126:E07
836	R0126:A12	877	R0126:E08
837	R0126:B01	878	R0126:E09
838	R0126:B03	879	R0126:E10
839	R0126:B04	880	R0126:E11
840	R0126:B05	881	R0126:E12
841	R0126:B06	882	R0126:F01
842	R0126:B07	883	R0126:F02
843	R0126:B08	884	R0126:F03
844	R0126:B09	885	R0126:F04
845	R0126:B11	886	R0126:F05
846	R0126:B12	887	R0126:F06
847	R0126:C01	888	R0126:F07
848	R0126:C02	889	R0126:F08
849	R0126:C03	890	R0126:F10
850	R0126:C05	891	R0126:F11
851	R0126:C06	892	R0126:F12
852	R0126:C07	893	R0126:G01
853	R0126:C08	894	R0126:G02
854	R0126:C09	895	R0126:G03
855	R0126:C10	896	R0126:G04
856	R0126:C11	897	R0126:G05
857	R0126:C12	898	R0126:G06
858	R0126:D01	899	R0126:G07
859	R0126:D02	900	R0126:G09
860	R0126:D03	901	R0126:G10
861	R0126:D04	902	R0126:G11
862	R0126:D05	903	R0126:G12
863	R0126:D06	904	R0126:H01
864	R0126:D07	905	R0126:H02
865	R0126:D08	906	R0126:H03
866	R0126:D09	907	R0126:H04

SEQ ID NO	CLONE IDENTIFIER	SEQ ID NO	CLONE IDENTIFIER
867	R0126:D10	908	R0126:H05
868	R0126:D11	909	R0126:H06

006230" 636T5360

Table 1B

SEQ ID NO	CLONE IDENTIFIER	SEQ ID NO	CLONE IDENTIFIER
910	R0126:H07	951	R0127:D10
911	R0126:H09	952	R0127:D11
912	R0126:H10	953	R0127:D12
913	R0126:H11	954	R0127:E02
914	R0127:A02	955	R0127:E03
915	R0127:A05	956	R0127:E04
916	R0127:A06	957	R0127:E05
917	R0127:A07	958	R0127:E06
918	R0127:A08	959	R0127:E07
919	R0127:A09	960	R0127:E08
920	R0127:A10	961	R0127:E09
921	R0127:A11	962	R0127:E10
922	R0127:A12	963	R0127:E11
923	R0127:B01	964	R0127:F01
924	R0127:B03	965	R0127:F02
925	R0127:B04	966	R0127:F03
926	R0127:B05	967	R0127:F04
927	R0127:B06	968	R0127:F05
928	R0127:B07	969	R0127:F06
929	R0127:B08	970	R0127:F07
930	R0127:B09	971	R0127:F08
931	R0127:B10	972	R0127:F10
932	R0127:B11	973	R0127:F11
933	R0127:B12	974	R0127:F12
934	R0127:C01	975	R0127:G01
935	R0127:C03	976	R0127:G02
936	R0127:C04	977	R0127:G03
937	R0127:C05	978	R0127:G04
938	R0127:C07	979	R0127:G05
939	R0127:C08	980	R0127:G06
940	R0127:C09	981	R0127:G07
941	R0127:C10	982	R0127:G08
942	R0127:C11	983	R0127:G09
943	R0127:D01	984	R0127:G10
944	R0127:D02	985	R0127:G11
945	R0127:D03	986	R0127:G12
946	R0127:D04	987	R0127:H01
947	R0127:D05	988	R0127:H02
948	R0127:D06	989	R0127:H03

SEQ ID NO	CLONE IDENTIFIER	SEQ ID NO	CLONE IDENTIFIER
949	R0127:D07	990	R0127:H04
950	R0127:D01	991	R0127:H05

005330" EST-3460

Table 1C

SEQ ID NO	CLONE IDENTIFIER	SEQ ID NO	CLONE IDENTIFIER
992	R0127:H06	1034	R0128:D11
993	R0127:H07	1035	R0128:D12
994	R0127:H08	1036	R0128:E01
995	R1027:H09	1037	R0128:E02
996	R1027:H10	1038	R0128:E03
997	R1027:H11	1039	R0128:E04
998	R1028:A02	1040	R0128:E05
999	R1028:A05	1041	R0128:E06
1000	R1028:A06	1042	R0128:E07
1001	R1028:A07	1043	R0128:E08
1002	R1028:A08	1044	R0128:E09
1003	R1028:A09	1045	R0128:E10
1004	R1028:A10	1046	R0128:E12
1005	R1028:B01	1047	R0128:F01
1006	R1028:B02	1048	R0128:F02
1007	R1028:B03	1049	R0128:F03
1008	R1028:B04	1050	R0128:F04
1009	R1028:B05	1051	R0128:F06
1010	R1028:B08	1052	R0128:F07
1011	R1028:B09	1053	R0128:F08
1012	R1028:B10	1054	R0128:F09
1013	R1028:B11	1055	R0128:F10
1014	R1028:B12	1056	R0128:F12
1015	R1028:C01	1057	R0128:G01
1016	R1028:C03	1058	R0128:G02
1017	R1028:C04	1059	R0128:G03
1018	R1028:C05	1060	R0128:G04
1019	R1028:C06	1061	R0128:G05
1020	R1028:C07	1062	R0128:G06
1021	R1028:C08	1063	R0128:G07
1022	R1028:C10	1064	R0128:G09
1023	R1028:C11	1065	R0128:G10
1024	R1028:C12	1066	R0128:G11
1025	R1028:D01	1067	R0128:G12
1026	R1028:D02	1068	R0128:H01
1027	R1028:D04	1069	R0128:H02
1028	R1028:D05	1070	R0128:H03
1029	R1028:D06	1071	R0128:H04
1030	R1028:D07	1072	R0128:H05
1031	R1028:D08	1073	R0128:H06

SEQ ID NO	CLONE IDENTIFIER	SEQ ID NO	CLONE IDENTIFIER
1032	R1028:D09	1074	R0128:H07
1033	R0128:D10	1075	R0128:H08

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Table 1D

SEQ ID NO	CLONE IDENTIFIER	SEQ ID NO	CLONE IDENTIFIER
1076	R0128:H09	1118	R0130:D12
1077	R0128:H10	1119	R0130:E01
1078	R0128:H11	1120	R0130:E02
1079	R0130:A02	1121	R0130:E03
1080	R0130:A05	1122	R0130:E04
1081	R0130:A06	1123	R0130:E05
1082	R0130:A08	1124	R0130:E06
1083	R0130:A09	1125	R0130:E07
1084	R0130:A10	1126	R0130:E08
1085	R0130:A11	1127	R0130:E09
1086	R0130:A12	1128	R0130:E10
1087	R0130:B01	1129	R0130:E11
1088	R0130:B02	1130	R0130:E12
1089	R0130:B03	1131	R0130:F02
1090	R0130:B04	1132	R0130:F03
1091	R0130:B05	1133	R0130:F05
1092	R0130:B06	1134	R0130:F06
1093	R0130:B08	1135	R0130:F07
1094	R0130:B09	1136	R0130:F08
1095	R0130:B10	1137	R0130:F09
1096	R0130:B11	1138	R0130:F10
1097	R0130:B12	1139	R0130:F11
1098	R0130:C02	1140	R0130:F12
1099	R0130:C03	1141	R0130:G01
1100	R0130:C04	1142	R0130:G02
1101	R0130:C05	1143	R0130:G03
1102	R0130:C06	1144	R0130:G04
1103	R0130:C07	1145	R0130:G05
1104	R0130:C08	1146	R0130:G06
1105	R0130:C09	1147	R0130:G07
1106	R0130:C10	1148	R0130:G08
1107	R0130:C11	1149	R0130:G09
1108	R0130:C12	1150	R0130:G10
1109	R0130:D02	1151	R0130:G11
1110	R0130:D03	1152	R0130:G12
1111	R0130:D04	1153	R0130:H01
1112	R0130:D05	1154	R0130:H02
1113	R0130:D06	1155	R0130:H04
1114	R0130:D07	1156	R0130:H05
1115	R0130:D09	1157	R0130:H06

SEQ ID NO	CLONE IDENTIFIER	SEQ ID NO	CLONE IDENTIFIER
1116	R0130:D10	1158	R0130:H07
1117	R0130:D11	1159	R0130:H08

1. Overall growth	
1970-75	1.02
1976-80	1.03
1981-85	1.04
1986-90	1.05
1991-95	1.06
2. Growth by region	
North	1.07
South	1.08
East	1.09
West	1.10
3. Growth by sector	
Manufacturing	1.11
Services	1.12
Construction	1.13
Agriculture	1.14
4. Growth by country	
USA	1.15
UK	1.16
France	1.17
Germany	1.18
Italy	1.19
Spain	1.20
Portugal	1.21
Greece	1.22
Belgium	1.23
Netherlands	1.24
Luxembourg	1.25
Austria	1.26
Switzerland	1.27
Sweden	1.28
Denmark	1.29
Finland	1.30
Poland	1.31
Czech Republic	1.32
Slovak Republic	1.33
Hungary	1.34
Romania	1.35
Bulgaria	1.36
Greece	1.37
Turkey	1.38
Yugoslavia	1.39
Croatia	1.40
Slovenia	1.41
Cyprus	1.42
Malta	1.43
Portugal	1.44
Spain	1.45
France	1.46
UK	1.47
USA	1.48
5. Growth by industry	
Automotive	1.49
Aerospace	1.50
Electronics	1.51
Chemicals	1.52
Metals	1.53
Textiles	1.54
Food	1.55
Drugs	1.56
Telecommunications	1.57
Energy	1.58
Transport	1.59
Healthcare	1.60
Education	1.61
Recreation	1.62
Real Estate	1.63
Finance	1.64
Insurance	1.65
Legal	1.66
Accounting	1.67
Consulting	1.68
Advertising	1.69
Public Relations	1.70
Media	1.71
Arts	1.72
Science	1.73
Technology	1.74
Environment	1.75
Energy	1.76
Transport	1.77
Healthcare	1.78
Education	1.79
Recreation	1.80
Real Estate	1.81
Finance	1.82
Insurance	1.83
Legal	1.84
Accounting	1.85
Consulting	1.86
Advertising	1.87
Public Relations	1.88
Media	1.89
Arts	1.90
Science	1.91
Technology	1.92
Environment	1.93
Energy	1.94
Transport	1.95
Healthcare	1.96
Education	1.97
Recreation	1.98
Real Estate	1.99
Finance	2.00
Insurance	2.01
Legal	2.02
Accounting	2.03
Consulting	2.04
Advertising	2.05
Public Relations	2.06
Media	2.07
Arts	2.08
Science	2.09
Technology	2.10
Environment	2.11
Energy	2.12
Transport	2.13
Healthcare	2.14
Education	2.15
Recreation	2.16
Real Estate	2.17
Finance	2.18
Insurance	2.19
Legal	2.20
Accounting	2.21
Consulting	2.22
Advertising	2.23
Public Relations	2.24
Media	2.25
Arts	2.26
Science	2.27
Technology	2.28
Environment	2.29
Energy	2.30
Transport	2.31
Healthcare	2.32
Education	2.33
Recreation	2.34
Real Estate	2.35
Finance	2.36
Insurance	2.37
Legal	2.38
Accounting	2.39
Consulting	2.40
Advertising	2.41
Public Relations	2.42
Media	2.43
Arts	2.44
Science	2.45
Technology	2.46
Environment	2.47
Energy	2.48
Transport	2.49
Healthcare	2.50
Education	2.51
Recreation	2.52
Real Estate	2.53
Finance	2.54
Insurance	2.55
Legal	2.56
Accounting	2.57
Consulting	2.58
Advertising	2.59
Public Relations	2.60
Media	2.61
Arts	2.62
Science	

Table 1E

SEQ ID NO	CLONE IDENTIFIER	SEQ ID NO	CLONE IDENTIFIER
1160	R0130:H09	1200	R0131:E01
1161	R0130:H10	1201	R0131:E02
1162	R0130:H11	1202	R0131:E03
1163	R0131:A02	1203	R0131:E04
1164	R0131:A05	1204	R0131:E06
1165	R0131:A06	1205	R0131:E07
1166	R0131:A07	1206	R0131:E08
1167	R0131:A08	1207	R0131:E10
1168	R0131:A09	1208	R0131:E11
1169	R0131:A11	1209	R0131:E12
1170	R0131:A12	1210	R0131:F02
1171	R0131:B02	1211	R0131:F04
1172	R0131:B03	1212	R0131:F05
1173	R0131:B04	1213	R0131:F06
1174	R0131:B05	1214	R0131:F07
1175	R0131:B07	1215	R0131:F08
1176	R0131:B08	1216	R0131:F09
1177	R0131:B09	1217	R0131:F10
1178	R0131:B10	1218	R0131:F11
1179	R0131:B11	1219	R0131:F12
1180	R0131:C01	1220	R0131:G01
1181	R0131:C02	1221	R0131:G02
1182	R0131:C03	1222	R0131:G03
1183	R0131:C04	1223	R0131:G04
1184	R0131:C06	1224	R0131:G05
1185	R0131:C07	1225	R0131:G06
1186	R0131:C08	1226	R0131:G07
1187	R0131:C10	1227	R0131:G08
1188	R0131:C11	1228	R0131:G09
1189	R0131:C12	1229	R0131:G10
1190	R0131:D02	1230	R0131:G11
1191	R0131:D03	1231	R0131:G12
1192	R0131:D04	1232	R0131:H01
1193	R0131:D05	1233	R0131:H02
1194	R0131:D06	1234	R0131:H05
1195	R0131:D07	1235	R0131:H06
1196	R0131:D09	1236	R0131:H07
1197	R0131:D10	1237	R0131:H08
1198	R0131:D11	1238	R0131:H09
1199	R0131:D12	1239	R0131:H11

Table 2:
Clone names for NSCLC-SQL1 and corresponding SEQ ID NOs

SEQ ID NO	CLONE IDENTIFIER	SEQ ID NO	CLONE IDENTIFIER
1240	Contig 54		
1241	Contig 55		
1242	Contig 57		
1243	Contig 58		
1244	Contig 60		
1245	Contig 62		
1246	Contig 63		
1247	Contig 64		
1248	Contig 65		
1249	Contig 66		
1250	Contig 67		
1251	Contig 68		
1252	Contig 69		
1253	Contig 70		
1254	Contig 71		
1255	Contig 72		
1256	Contig 73		
1257	Contig 74		
1258	Contig 75		
1259	Contig 77		
1260	Contig 78		
1261	Contig 79		
1262	Contig 80		
1263	Contig 81		
1264	Contig 83		
1265	Contig 84		
1266	Contig 86		
1267	Contig 87		
1268	Contig 88		
1269	Contig 89		
1270	Contig 90		
1271	Contig 91		
1272	Contig 92		
1273	Contig 94		
1274	Contig 95		
1275	Contig 96		
1276	Contig 97		
1277	Contig 98		

SEQ ID NO	CLONE IDENTIFIER	SEQ ID NO	CLONE IDENTIFIER
1278	Contig 99		
1279	Contig 100		

0061563-082900

Table 3:
Clone names for NSCLC-SCLI and corresponding SEQ ID NOs

SEQ ID NO	CLONE IDENTIFIER	SEQ ID NO	CLONE IDENTIFIER
1280	Contig 38	1320	Contig 82
1281	Contig 39		
1282	Contig 40		
1283	Contig 41		
1284	Contig 42		
1285	Contig 43		
1286	Contig 44		
1287	Contig 45		
1288	Contig 46		
1289	Contig 47		
1290	Contig 48		
1291	Contig 49		
1292	Contig 51		
1293	Contig 52		
1294	Contig 53		
1295	Contig 54		
1296	Contig 55		
1297	Contig 56		
1298	Contig 57		
1299	Contig 58		
1300	Contig 59		
1301	Contig 60		
1302	Contig 62		
1303	Contig 63		
1304	Contig 64		
1305	Contig 65		
1306	Contig 66		
1307	Contig 67		
1308	Contig 68		
1309	Contig 69		
1310	Contig 70		
1311	Contig 72		
1312	Contig 73		
1313	Contig 75		
1314	Contig 76		
1315	Contig 77		
1316	Contig 78		
1317	Contig 79		

SEQ ID NO	CLONE IDENTIFIER	SEQ ID NO	CLONE IDENTIFIER
1318	Contig 80		
1319	Contig 81		

005280 032900

Table 4A:
Clone names for NSCLC-SCL3-SCL4 and corresponding SEQ ID NOs

SEQ ID NO	CLONE IDENTIFIER	SEQ ID NO	CLONE IDENTIFIER
1321	Contig 94	1363	Contig 136
1322	Contig 95	1364	Contig 137
1323	Contig 96	1365	Contig 138
1324	Contig 97	1366	Contig 139
1325	Contig 98	1367	Contig 140
1326	Contig 99	1368	Contig 141
1327	Contig 100	1369	Contig 142
1328	Contig 101	1370	Contig 143
1329	Contig 102	1371	Contig 144
1330	Contig 103	1372	Contig 145
1331	Contig 104	1373	Contig 146
1332	Contig 105	1374	Contig 147
1333	Contig 106	1375	Contig 148
1334	Contig 107	1376	Contig 149
1335	Contig 108	1377	Contig 150
1336	Contig 109	1378	Contig 151
1337	Contig 110	1379	Contig 152
1338	Contig 111	1380	Contig 153
1339	Contig 112	1381	Contig 154
1340	Contig 113	1382	Contig 155
1341	Contig 114	1383	Contig 156
1342	Contig 115	1384	Contig 157
1343	Contig 116	1385	Contig 158
1344	Contig 117	1386	Contig 159
1345	Contig 118	1387	Contig 160
1346	Contig 119	1388	Contig 161
1347	Contig 120	1389	Contig 162
1348	Contig 121	1390	Contig 163
1349	Contig 122	1391	Contig 164
1350	Contig 123	1392	Contig 165
1351	Contig 124	1393	Contig 166
1352	Contig 125	1394	Contig 167
1353	Contig 126	1395	Contig 168
1354	Contig 127	1396	Contig 169
1355	Contig 128	1397	Contig 170
1356	Contig 129	1398	Contig 171
1357	Contig 130	1399	Contig 172
1358	Contig 131	1400	Contig 173
1359	Contig 132	1401	Contig 174

Table 4B:
Clone names for NSCLC-SCL3-SCL4 and corresponding SEQ ID NOs

SEQ ID NO	CLONE IDENTIFIER	SEQ ID NO	CLONE IDENTIFIER
1404	Contig 177		
1405	Contig 178		
1406	Contig 179		
1407	Contig 180		
1408	Contig 181		
1409	Contig 182		
1410	Contig 183		
1411	Contig 184		
1412	Contig 185		
1413	Contig 186		
1414	Contig 187		

006280 "EST5960

Table 5:
Clone names for SCLC-SQL1 and corresponding SEQ ID NOs

SEQ ID NO	CLONE IDENTIFIER	SEQ ID NO	CLONE IDENTIFIER
1415	Contig 17		
1416	Contig 18		
1417	Contig 20		
1418	Contig 23		
1419	Contig 24		
1420	Contig 25		
1421	Contig 26		
1422	Contig 27		
1423	Contig 28		
1424	Contig 29		
1425	Contig 30		
1426	Contig 31		
1427	Contig 20		
1428	Contig 21		
1429	Contig 22		
1430	Contig 23		
1431	Contig 24		
1432	Contig 25		
1433	Contig 26		
1434	Contig 27		
1435	Contig 28		
1436	Contig 29		
1437	Contig 30		
1438	Contig 31		
1439	Contig 32		
1440	Contig 33		
1441	Contig 34		
1442	Contig 35		
1443	Contig 36		
1444	Contig 37		
1445	Contig 38		

006230" E3515950

Table 6A:
Clone names for SCLC-SCL3-SCL4 and corresponding SEQ ID NOs

SEQ ID NO	CLONE IDENTIFIER	SEQ ID NO	CLONE IDENTIFIER
1446	Contig 116	1488	Contig 160
1447	Contig 117	1489	Contig 161
1448	Contig 118	1490	Contig 162
1449	Contig 119	1491	Contig 163
1450	Contig 120	1492	Contig 164
1451	Contig 122	1493	Contig 165
1452	Contig 123	1494	Contig 166
1453	Contig 124	1495	Contig 167
1454	Contig 125	1496	Contig 168
1455	Contig 126	1497	Contig 169
1456	Contig 127	1498	Contig 170
1457	Contig 128	1499	Contig 171
1458	Contig 129	1500	Contig 172
1459	Contig 130	1501	Contig 173
1460	Contig 131	1502	Contig 174
1461	Contig 132	1503	Contig 175
1462	Contig 133	1504	Contig 176
1463	Contig 135	1505	Contig 177
1464	Contig 136	1506	Contig 178
1465	Contig 137	1507	Contig 179
1466	Contig 138	1508	Contig 181
1467	Contig 139 (L985P)	1509	Contig 182
1468	Contig 140	1510	Contig 183
1469	Contig 141	1511	Contig 184
1470	Contig 142	1512	Contig 185
1471	Contig 143	1513	Contig 186
1472	Contig 144	1514	Contig 187
1473	Contig 145	1515	Contig 189
1474	Contig 146	1516	Contig 190
1475	Contig 147	1517	Contig 191
1476	Contig 148	1518	Contig 192
1477	Contig 149	1519	Contig 193
1478	Contig 150	1520	Contig 194
1479	Contig 151	1521	Contig 195
1480	Contig 152	1522	Contig 196
1481	Contig 153	1523	Contig 197
1482	Contig 154	1524	Contig 198
1483	Contig 155	1525	Contig 199
1484	Contig 156	1526	Contig 200

SEQ ID NO	CLONE IDENTIFIER	SEQ ID NO	CLONE IDENTIFIER
1485	Contig 157	1527	Contig 201
1486	Contig 158	1528	Contig 202
1487	Contig 159		

006280" E84T2960

Table 6B:
Clone names for SCLC-SCL3-SCL4 and corresponding SEQ ID NOs

SEQ ID NO	CLONE IDENTIFIER	SEQ ID NO	CLONE IDENTIFIER
1529	Contig 203		
1530	Contig 204		
1531	Contig 205		
1532	Contig 206		
1533	Contig 207		
1534	Contig 208		
1535	Contig 209		
1536	Contig 210		
1537	Contig 211		
1538	Contig 212		
1539	Contig 213		
1540	Contig 214		
1541	Contig 215		
1542	Contig 216		
1543	Contig 217		
1544	Contig 218		
1545	Contig 219		
1546	Contig 220		
1547	Contig 221		
1548	Contig 222		
1549	Contig 223		
1550	Contig 224		
1551	Contig 225		
1552	Contig 226		
1553	Contig 227		
1554	Contig 228		
1555	Contig 229		
1556	Contig 230		
1557	Contig 231		
1558	Contig 232		
1559	Contig 233		
1560	Contig 234		
1561	Contig 235		
1562	Contig 236		
1563	Contig 237		



SEQ ID NO	CLONE IDENTIFIER	SEQ ID NO	CLONE IDENTIFIER
1564	R0124:E05	1609	R0129:D09
1565	R0124:E06	1610	R0129:D10
1566	R0124:E08	1611	R0129:D11
1567	R0124:F07	1612	R0129:E02
1568	R0124:F08	1613	R0129:E03
1569	R0124:F09	1614	R0129:E04
1570	R0124:G04	1615	R0129:E05
1571	R0129:A02	1616	R0129:E06
1572	R0129:A03	1617	R0129:E07
1573	R0129:A06	1618	R0129:E08
1574	R0129:A07	1619	R0129:E09
1575	R0129:A08	1620	R0129:E11
1576	R0129:A09	1621	R0129:E12
1577	R0129:A10	1622	R0129:F01
1578	R0129:A11	1623	R0129:F02
1579	R0129:A12	1624	R0129:F03
1580	R0129:B02	1625	R0129:F04
1581	R0129:B03	1626	R0129:F06
1582	R0129:B04	1627	R0129:F07
1583	R0129:B05	1628	R0129:F08
1584	R0129:B06	1629	R0129:F09
1585	R0129:B07	1630	R0129:F10
1586	R0129:B08	1631	R0129:F11
1587	R0129:B09	1632	R0129:F12
1588	R0129:B10	1633	R0129:G01
1589	R0129:B11	1634	R0129:G02
1590	R0129:B12	1635	R0129:G03
1591	R0129:C01	1636	R0129:G04
1592	R0129:C02	1637	R0129:G05
1593	R0129:C03	1638	R0129:G06
1594	R0129:C04	1639	R0129:G07
1595	R0129:C06	1640	R0129:G08
1596	R0129:C07	1641	R0129:G09
1597	R0129:C08	1642	R0129:G10
1598	R0129:C09	1643	R0129:G11
1599	R0129:C10	1644	R0129:G12
1600	R0129:C11	1645	R0129:H01
1601	R0129:C12	1646	R0129:H02
1602	R0129:D01	1647	R0129:H03
1603	R0129:D03	1648	R0129:H04

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Table 8.

SEQ ID NO	CLONE IDENTIFIER	SEQ ID NO	CLONE IDENTIFIER
1654	26484		
1655	26496		
1656	26517		
1657	26531		
1658	26022		
1659	26026		
1660	26810		
1661	26815		
1662	26869		
1663	26883		
1664	26902		

SEQ ID NO:1667 is the protein sequence of expressed recombinant

5 L7548S.

SEQ ID NO:1668 is the cDNA sequence of expressed recombinant L7548S.

SEQ ID NO:1669 is the extended cDNA sequence of clone #18971

(L801P).

SEQ ID NO:1670 is the amino acid sequence of open reading frame ORF4

10 encoded by SEQ ID NO:1669.

SEQ ID NO:1671 is the amino acid sequence of open reading frame ORF5

encoded by SEQ ID NO:1669.

SEQ ID NO:1672 is the amino acid sequence of open reading frame ORF6

encoded by SEQ ID NO:1669.

SEQ ID NO:1673 is the amino acid sequence of open reading frame ORF7

15 encoded by SEQ ID NO:1669.

SEQ ID NO:1674 is the amino acid sequence of open reading frame ORF8

encoded by SEQ ID NO:1669.

SEQ ID NO:1675 is the amino acid sequence of open reading frame ORF9

20 encoded by SEQ ID NO:1669.

SEQ ID NO:1676 is the extended cDNA for contig 139 (SEQ ID NO:1467),

also known as L985P.

SEQ ID NO:1677 is the L985P amino acid sequence encoded by SEQ ID NO: 1676.

SEQ ID NO: 1678 is the amino acid sequence of open reading frame ORF5X of SEQ ID NO:1669.

- 5 SEQ ID NO: 1679 is the amino acid sequence of an open reading frame for contig 139 (SEQ ID NO:1467).

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is generally directed to compositions and methods for using the compositions, for example in the therapy and diagnosis of cancer, such as lung cancer. Certain illustrative compositions described herein include lung tumor polypeptides, polynucleotides encoding such polypeptides, binding agents such as antibodies, antigen presenting cells (APCs) and/or immune system cells (*e.g.*, T cells). A "lung tumor protein," as the term is used herein, refers generally to a protein that is expressed in lung tumor cells at a level that is at least two fold, and preferably at least five fold, greater than the level of expression in a normal tissue, as determined using a representative assay provided herein. Certain lung tumor proteins are tumor proteins that react detectably (within an immunoassay, such as an ELISA or Western blot) with antisera of a patient afflicted with lung cancer.

20 Therefore, in accordance with the above, and as described further below, the present invention provides illustrative polynucleotide compositions having sequences set forth in SEQ ID NO: 1, 11-13, 15, 20, 23-27, 29, 30, 33, 34, 39, 41, 43-46, 51, 52, 57, 58, 60, 62, 65-67, 69-71, 74, 76, 79, 80, 84, 86, 89-92, 95, 97, 98, 101, 110, 111, 113-119, 121-128, 130-134, 136, 138, 139, 141, 143, 146-151, 153, 154, 157-160, 162-164, 167-178, 25 180, 181, 183, 186-190, 192, 193, 195-220, 224, 226-231, 234, 236, 237, 240, 241, 244-246, 248, 254, 255, 261, 262, 266, 270, 275, 280, 282, 283, 288, 289, 290, 292, 295, 301, 303, 304, 309, 311, 341-782, 784, 785, 790, 792, 794, 796, 800-804, 807, 808, 810-826, 1240, 1243, 1247, 1269, 1272, 1280, 1283, 1285, 1286, 1289, 1300, 1309, 1318, 1319,

10 POLYNUCLEOTIDE COMPOSITIONS

As will be understood by those skilled in the art, the DNA segments of this invention can include genomic sequences, extra-genomic and plasmid-encoded sequences and smaller engineered gene segments that express, or may be adapted to express, proteins, polypeptides, peptides and the like. Such segments may be naturally isolated, or modified synthetically by the hand of man.

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isolated, and does not exclude genes or coding regions later added to the segment by the hand of man.

As will be recognized by the skilled artisan, polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. RNA molecules include HnRNA molecules, which contain introns and correspond to a DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain introns. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present invention, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

Polynucleotides may comprise a native sequence (*i.e.*, an endogenous sequence that encodes a lung tumor protein or a portion thereof) or may comprise a variant, or a biological or antigenic functional equivalent of such a sequence. Polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions, as further described below, preferably such that the immunogenicity of the encoded polypeptide is not diminished, relative to a native tumor protein. The effect on the immunogenicity of the encoded polypeptide may generally be assessed as described herein. The term "variants" also encompasses homologous genes of xenogenic origin.

When comparing polynucleotide or polypeptide sequences, two sequences are said to be "identical" if the sequence of nucleotides or amino acids in the two sequences is the same when aligned for maximum correspondence, as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment

- schemes described in the following references: Dayhoff, M.O. (1978) A model of evolutionary change in proteins – Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J. (1990) Unified
- 5 Approach to Alignment and Phylogenesis pp. 626-645 *Methods in Enzymology* vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M. (1989) *CABIOS* 5:151-153; Myers, E.W. and Muller W. (1988) *CABIOS* 4:11-17; Robinson, E.D. (1971) *Comb. Theor* 11:105; Santou, N. Nes, M. (1987) *Mol. Biol. Evol.* 4:406-425; Sneath, P.H.A. and Sokal, R.R. (1973) *Numerical Taxonomy – the Principles and Practice of*
- 10 *Numerical Taxonomy*, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman, D.J. (1983) *Proc. Natl. Acad., Sci. USA* 80:726-730.

- Alternatively, optimal alignment of sequences for comparison may be conducted by the local identity algorithm of Smith and Waterman (1981) *Add. APL. Math* 2:482, by the identity alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.*
- 15 48:443, by the search for similarity methods of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. USA* 85: 2444, by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by inspection.

- One preferred example of algorithms that are suitable for determining
- 20 percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.* (1977) *Nucl. Acids Res.* 25:3389-3402 and Altschul *et al.* (1990) *J. Mol. Biol.* 215:403-410, respectively. BLAST and BLAST 2.0 can be used, for example with the parameters described herein, to determine percent sequence identity for the polynucleotides and polypeptides of the invention. Software for
- 25 performing BLAST analyses is publicly available through the National Center for Biotechnology Information. In one illustrative example, cumulative scores can be calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). For amino acid sequences, a scoring matrix can be used to calculate the cumulative score.

Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1989) *Proc. Natl. Acad. Sci. USA* 89:10915) alignments, (B) of 50, expectation (E) of 10, M=5, N=-4 and a comparison of both strands.

- 10 Preferably, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which
- 15 does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (*i.e.*, the window size) and multiplying the results by
- 20 100 to yield the percentage of sequence identity.

Therefore, the present invention encompasses polynucleotide and polypeptide sequences having substantial identity to the sequences disclosed herein, for example those comprising at least 50% sequence identity, preferably at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% or higher, sequence

25 identity compared to a polynucleotide or polypeptide sequence of this invention using the methods described herein, (*e.g.*, BLAST analysis using standard parameters, as described below). One skilled in this art will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide

sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like.

In additional embodiments, the present invention provides isolated polynucleotides and polypeptides comprising various lengths of contiguous stretches of sequence identical to or complementary to one or more of the sequences disclosed herein. For example, polynucleotides are provided by this invention that comprise at least about 15, 20, 30, 40, 50, 75, 100, 150, 200, 300, 400, 500 or 1000 or more contiguous nucleotides of one or more of the sequences disclosed herein as well as all intermediate lengths there between. It will be readily understood that "intermediate lengths", in this context, means any length between the quoted values, such as 16, 17, 18, 19, *etc.*; 21, 22, 23, *etc.*; 30, 31, 32, *etc.*; 50, 51, 52, 53, *etc.*; 100, 101, 102, 103, *etc.*; 150, 151, 152, 153, *etc.*; including all integers through 200-500; 500-1,000, and the like.

The polynucleotides of the present invention, or fragments thereof, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol. For example, illustrative DNA segments with total lengths of about 10,000, about 5000, about 3000, about 2,000, about 1,000, about 500, about 200, about 100, about 50 base pairs in length, and the like, (including all intermediate lengths) are contemplated to be useful in many implementations of this invention.

In other embodiments, the present invention is directed to polynucleotides that are capable of hybridizing under moderately stringent conditions to a polynucleotide sequence provided herein, or a fragment thereof, or a complementary sequence thereof. Hybridization techniques are well known in the art of molecular biology. For purposes of illustration, suitable moderately stringent conditions for testing the hybridization of a polynucleotide of this invention with other polynucleotides include prewashing in a

solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5 X SSC, overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS.

Moreover, it will be appreciated by those of ordinary skill in the art that, as
5 a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present invention. Further, alleles of the genes comprising the polynucleotide sequences provided
10 herein are within the scope of the present invention. Alleles are endogenous genes that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides. The resulting mRNA and protein may, but need not, have an altered structure or function. Alleles may be identified using standard techniques (such as hybridization, amplification and/or database sequence comparison).

15 PROBES AND PRIMERS

In other embodiments of the present invention, the polynucleotide sequences provided herein can be advantageously used as probes or primers for nucleic acid hybridization. As such, it is contemplated that nucleic acid segments that comprise a sequence region of at least about 15 nucleotide long contiguous sequence that has the same
20 sequence as, or is complementary to, a 15 nucleotide long contiguous sequence disclosed herein will find particular utility. Longer contiguous identical or complementary sequences, *e.g.*, those of about 20, 30, 40, 50, 100, 200, 500, 1000 (including all intermediate lengths) and even up to full length sequences will also be of use in certain embodiments.

25 The ability of such nucleic acid probes to specifically hybridize to a sequence of interest will enable them to be of use in detecting the presence of complementary sequences in a given sample. However, other uses are also envisioned,

such as the use of the sequence information for the preparation of mutant species primers, or primers for use in preparing other genetic constructions.

Polynucleotide molecules having sequence regions consisting of contiguous nucleotide stretches of 10-14, 15-20, 30, 50, or even of 100-200 nucleotides or so (including intermediate lengths as well), identical or complementary to a polynucleotide sequence disclosed herein, are particularly contemplated as hybridization probes for use in, *e.g.*, Southern and Northern blotting. This would allow a gene product, or fragment thereof, to be analyzed, both in diverse cell types and also in various bacterial cells. The total size of fragment, as well as the size of the complementary stretch(es), will ultimately depend on the intended use or application of the particular nucleic acid segment. Smaller fragments will generally find use in hybridization embodiments, wherein the length of the contiguous complementary region may be varied, such as between about 15 and about 100 nucleotides, but larger contiguous complementarity stretches may be used, according to the length complementary sequences one wishes to detect.

The use of a hybridization probe of about 15-25 nucleotides in length allows the formation of a duplex molecule that is both stable and selective. Molecules having contiguous complementary sequences over stretches greater than 15 bases in length are generally preferred, though, in order to increase stability and selectivity of the hybrid, and thereby improve the quality and degree of specific hybrid molecules obtained. One will generally prefer to design nucleic acid molecules having gene-complementary stretches of 15 to 25 contiguous nucleotides, or even longer where desired.

Hybridization probes may be selected from any portion of any of the sequences disclosed herein. All that is required is to review the sequence set forth in SEQ ID NO: 1, 11-13, 15, 20, 23-27, 29, 30, 33, 34, 39, 41, 43-46, 51, 52, 57, 58, 60, 62, 65-67, 69-71, 74, 76, 79, 80, 84, 86, 89-92, 95, 97, 98, 101, 110, 111, 113-119, 121-128, 130-134, 136, 138, 139, 141, 143, 146-151, 153, 154, 157-160, 162-164, 167-178, 180, 181, 183, 186-190, 192, 193, 195-220, 224, 226-231, 234, 236, 237, 240, 241, 244-246, 248, 254, 255, 261, 262, 266, 270, 275, 280, 282, 283, 288, 289, 290, 292, 295, 301, 303, 304, 309, 311, 341-782, 784, 785, 790, 792, 794, 796, 800-804, 807, 808, 810-826, 828-1664, 1669

and 1676, or to any continuous portion of the sequence, from about 15-25 nucleotides in length up to and including the full length sequence, that one wishes to utilize as a probe or primer. The choice of probe and primer sequences may be governed by various factors. For example, one may wish to employ primers from towards the termini of the total
5 sequence.

Small polynucleotide segments or fragments may be readily prepared by, for example, directly synthesizing the fragment by chemical means, as is commonly practiced using an automated oligonucleotide synthesizer. Also, fragments may be obtained by application of nucleic acid reproduction technology, such as the PCR™ technology of U. S.
10 Patent 4,683,202 (incorporated herein by reference), by introducing selected sequences into recombinant vectors for recombinant production, and by other recombinant DNA techniques generally known to those of skill in the art of molecular biology.

The nucleotide sequences of the invention may be used for their ability to selectively form duplex molecules with complementary stretches of the entire gene or gene
15 fragments of interest. Depending on the application envisioned, one will typically desire to employ varying conditions of hybridization to achieve varying degrees of selectivity of probe towards target sequence. For applications requiring high selectivity, one will typically desire to employ relatively stringent conditions to form the hybrids, *e.g.*, one will select relatively low salt and/or high temperature conditions, such as provided by a salt
20 concentration of from about 0.02 M to about 0.15 M salt at temperatures of from about 50°C to about 70°C. Such selective conditions tolerate little, if any, mismatch between the probe and the template or target strand, and would be particularly suitable for isolating related sequences.

Of course, for some applications, for example, where one desires to prepare
25 mutants employing a mutant primer strand hybridized to an underlying template, less stringent (reduced stringency) hybridization conditions will typically be needed in order to allow formation of the heteroduplex. In these circumstances, one may desire to employ salt conditions such as those of from about 0.15 M to about 0.9 M salt, at temperatures ranging from about 20°C to about 55°C. Cross-hybridizing species can thereby be readily

identified as positively hybridizing signals with respect to control hybridizations. In any case, it is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide, which serves to destabilize the hybrid duplex in the same manner as increased temperature. Thus, hybridization conditions can be readily manipulated, and thus will generally be a method of choice depending on the desired results.

POLYNUCLEOTIDE IDENTIFICATION AND CHARACTERIZATION

Polynucleotides may be identified, prepared and/or manipulated using any of a variety of well established techniques. For example, a polynucleotide may be identified, as described in more detail below, by screening a microarray of cDNAs for tumor-associated expression (*i.e.*, expression that is at least two fold greater in a tumor than in normal tissue, as determined using a representative assay provided herein). Such screens may be performed, for example, using a Synteni microarray (Palo Alto, CA) according to the manufacturer's instructions (and essentially as described by Schena *et al.*, *Proc. Natl. Acad. Sci. USA* 93:10614-10619, 1996 and Heller *et al.*, *Proc. Natl. Acad. Sci. USA* 94:2150-2155, 1997). Alternatively, polynucleotides may be amplified from cDNA prepared from cells expressing the proteins described herein, such as lung tumor cells. Such polynucleotides may be amplified via polymerase chain reaction (PCR). For this approach, sequence-specific primers may be designed based on the sequences provided herein, and may be purchased or synthesized.

An amplified portion of a polynucleotide of the present invention may be used to isolate a full length gene from a suitable library (*e.g.*, a lung tumor cDNA library) using well known techniques. Within such techniques, a library (cDNA or genomic) is screened using one or more polynucleotide probes or primers suitable for amplification. Preferably, a library is size-selected to include larger molecules. Random primed libraries may also be preferred for identifying 5' and upstream regions of genes. Genomic libraries are preferred for obtaining introns and extending 5' sequences.

Alternatively, there are numerous amplification techniques for obtaining a full length coding sequence from a partial cDNA sequence. Within such techniques, amplification is generally performed via PCR. Any of a variety of commercially available kits may be used to perform the amplification step. Primers may be designed using, for example, software well known in the art. Primers are preferably 22-30 nucleotides in length, have a GC content of at least 50% and anneal to the target sequence at temperatures of about 68°C to 72°C. The amplified region may be sequenced as described above, and overlapping sequences assembled into a contiguous sequence.

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same linker primer and a second primer specific to the known region. A variation on this procedure, which employs two primers that initiate extension in opposite directions from the known sequence, is described in WO 96/38591. Another such technique is known as "rapid amplification of cDNA ends" or RACE. This technique involves the use of an
 5 internal primer and an external primer, which hybridizes to a polyA region or vector sequence, to identify sequences that are 5' and 3' of a known sequence. Additional techniques include capture PCR (Lagerstrom *et al.*, *PCR Methods Applic.* 1:111-19, 1991) and walking PCR (Parker *et al.*, *Nucl. Acids. Res.* 19:3055-60, 1991). Other methods employing amplification may also be employed to obtain a full length cDNA sequence.

10 In certain instances, it is possible to obtain a full length cDNA sequence by analysis of sequences provided in an expressed sequence tag (EST) database, such as that available from GenBank. Searches for overlapping ESTs may generally be performed using well known programs (*e.g.*, NCBI BLAST searches), and such ESTs may be used to generate a contiguous full length sequence. Full length DNA sequences may also be
 15 obtained by analysis of genomic fragments.

POLYNUCLEOTIDE EXPRESSION IN HOST CELLS

In other embodiments of the invention, polynucleotide sequences or fragments thereof which encode polypeptides of the invention, or fusion proteins or functional equivalents thereof, may be used in recombinant DNA molecules to direct
 20 expression of a polypeptide in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences that encode substantially the same or a functionally equivalent amino acid sequence may be produced and these sequences may be used to clone and express a given polypeptide.

As will be understood by those of skill in the art, it may be advantageous in
 25 some instances to produce polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce a

recombinant RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences may be ligated to a heterologous sequence to encode a fusion protein. For example, to screen peptide libraries for inhibitors of polypeptide activity, it may be useful to encode a chimeric protein that can be recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the polypeptide-encoding sequence and the heterologous protein sequence, so that the polypeptide may be cleaved and purified away from the heterologous moiety.

Sequences encoding a desired polypeptide may be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers, M. H. *et al.* (1980) *Nucl. Acids Res. Symp. Ser.* 215-223, Horn, T. *et al.* (1980) *Nucl. Acids Res. Symp. Ser.* 225-232). Alternatively, the protein itself may be produced using chemical methods to synthesize the amino acid sequence of a polypeptide, or a portion thereof. For example, peptide synthesis can be performed using various solid-phase techniques (Roberge, J. Y. *et al.* (1995) *Science* 269:202-204) and automated synthesis may be achieved, for example, using the ABI 431A Peptide Synthesizer (Perkin Elmer, Palo Alto, CA).

A newly synthesized peptide may be substantially purified by preparative high performance liquid chromatography (*e.g.*, Creighton, T. (1983) *Proteins, Structures and Molecular Principles*, WH Freeman and Co., New York, N.Y.) or other comparable

techniques available in the art. The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure). Additionally, the amino acid sequence of a polypeptide, or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with sequences from
 5 other proteins, or any part thereof, to produce a variant polypeptide.

In order to express a desired polypeptide, the nucleotide sequences encoding the polypeptide, or functional equivalents, may be inserted into appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those
 10 skilled in the art may be used to construct expression vectors containing sequences encoding a polypeptide of interest and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. Such techniques are described in Sambrook, J. *et al.* (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press,
 15 Plainview, N.Y., and Ausubel, F. M. *et al.* (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York. N.Y.

A variety of expression vector/host systems may be utilized to contain and express polynucleotide sequences. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA
 20 expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (*e.g.*, baculovirus); plant cell systems transformed with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmids); or animal cell systems.

25 The "control elements" or "regulatory sequences" present in an expression vector are those non-translated regions of the vector--enhancers, promoters, 5' and 3' untranslated regions--which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation

elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the PBLUESCRIPT phagemid (Stratagene, La Jolla, Calif.) or PSPORT1 plasmid (Gibco BRL, Gaithersburg, MD) and the like may be used. In mammalian cell systems, promoters from
5 mammalian genes or from mammalian viruses are generally preferred. If it is necessary to generate a cell line that contains multiple copies of the sequence encoding a polypeptide, vectors based on SV40 or EBV may be advantageously used with an appropriate selectable marker.

In bacterial systems, a number of expression vectors may be selected
10 depending upon the use intended for the expressed polypeptide. For example, when large quantities are needed, for example for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be used. Such vectors include, but are not limited to, the multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene), in which the sequence encoding the polypeptide of
15 interest may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of .beta.-galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke, G. and S. M. Schuster (1989) *J. Biol. Chem.* 264:5503-5509); and the like. pGEX Vectors (Promega, Madison, Wis.) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such
20 fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems may be designed to include heparin, thrombin, or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

25 In the yeast, *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH may be used. For reviews, see Ausubel *et al.* (supra) and Grant *et al.* (1987) *Methods Enzymol.* 153:516-544.

In cases where plant expression vectors are used, the expression of sequences encoding polypeptides may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV may be used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) *EMBO J.* 6:307-311. Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used (Coruzzi, G. *et al.* (1984) *EMBO J.* 3:1671-1680; Broglie, R. *et al.* (1984) *Science* 224:838-843; and Winter, J. *et al.* (1991) *Results Probl. Cell Differ.* 17:85-105). These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (see, for example, Hobbs, S. or Murry, L. E. in McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York, N.Y.; pp. 191-196).

An insect system may also be used to express a polypeptide of interest. For example, in one such system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. The sequences encoding the polypeptide may be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of the polypeptide-encoding sequence will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses may then be used to infect, for example, *S. frugiperda* cells or *Trichoplusia* larvae in which the polypeptide of interest may be expressed (Engelhard, E. K. *et al.* (1994) *Proc. Natl. Acad. Sci.* 91 :3224-3227).

In mammalian host cells, a number of viral-based expression systems are generally available. For example, in cases where an adenovirus is used as an expression vector, sequences encoding a polypeptide of interest may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain a viable virus which is capable of expressing the polypeptide in infected host cells (Logan, J. and Shenk, T. (1984) *Proc. Natl. Acad. Sci.* 81:3655-3659). In addition,

transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Specific initiation signals may also be used to achieve more efficient translation of sequences encoding a polypeptide of interest. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding the polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous translational control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used, such as those described in the literature (Scharf, D. *et al.* (1994) *Results Probl. Cell Differ.* 20:125-162).

In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, HEK293, and WI38, which have specific cellular machinery and characteristic mechanisms for such post-translational activities, may be chosen to ensure the correct modification and processing of the foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is generally preferred. For example, cell lines which stably express a polynucleotide of interest may be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells

may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated
 5 using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler, M. *et al.* (1977) *Cell* 11:223-32) and adenine phosphoribosyltransferase (Lowy, I. *et al.* (1990) *Cell* 22:817-23) genes which can be employed in tk.sup.- or aprt.sup.- cells,
 10 respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dhfr which confers resistance to methotrexate (Wigler, M. *et al.* (1980) *Proc. Natl. Acad. Sci.* 77:3567-70); npt, which confers resistance to the aminoglycosides, neomycin and G-418 (Colbere-Garapin, F. *et al.* (1981) *J. Mol. Biol.* 150:1-14); and als or pat, which confer resistance to chlorsulfuron and phosphinotricin
 15 acetyltransferase, respectively (Murry, *supra*). Additional selectable genes have been described, for example, trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine (Hartman, S. C. and R. C. Mulligan (1988) *Proc. Natl. Acad. Sci.* 85:8047-51). Recently, the use of visible markers has gained popularity with such markers as anthocyanins, beta-glucuronidase and its
 20 substrate GUS, and luciferase and its substrate luciferin, being widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes, C. A. *et al.* (1995) *Methods Mol. Biol.* 55:121-131).

Although the presence/absence of marker gene expression suggests that the
 25 gene of interest is also present, its presence and expression may need to be confirmed. For example, if the sequence encoding a polypeptide is inserted within a marker gene sequence, recombinant cells containing sequences can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a polypeptide-encoding sequence under the control of a single promoter. Expression of the marker gene

in response to induction or selection usually indicates expression of the tandem gene as well.

Alternatively, host cells which contain and express a desired polynucleotide sequence may be identified by a variety of procedures known to those of skill in the art.

- 5 These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein.

- A variety of protocols for detecting and measuring the expression of polynucleotide-encoded products, using either polyclonal or monoclonal antibodies specific for the product are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on a given polypeptide may be preferred for some applications, but a competitive binding assay may also be employed. These and other assays are described, among other places, in Hampton, R. *et al.* (1990; Serological Methods, a Laboratory Manual, APS Press, St Paul, Minn.) and Maddox, D. E. *et al.* (1983; *J. Exp. Med.* 158:1211-1216).
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- A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides include oligolabeling, nick translation, end-labeling or PCR amplification using a labeled nucleotide. Alternatively, the sequences, or any portions thereof may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits. Suitable reporter molecules or labels, which may be used include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.
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Host cells transformed with a polynucleotide sequence of interest may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides of the invention may be designed to contain signal sequences which direct secretion of the encoded polypeptide through a prokaryotic or eukaryotic cell membrane. Other recombinant constructions may be used to join sequences encoding a polypeptide of interest to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). The inclusion of cleavable linker sequences such as those specific for Factor XA or enterokinase (Invitrogen, San Diego, Calif.) between the purification domain and the encoded polypeptide may be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing a polypeptide of interest and a nucleic acid encoding 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromatography) as described in Porath, J. *et al.* (1992, *Prot. Exp. Purif.* 3:263-281) while the enterokinase cleavage site provides a means for purifying the desired polypeptide from the fusion protein. A discussion of vectors which contain fusion proteins is provided in Kroll, D. J. *et al.* (1993; *DNA Cell Biol.* 12:441-453).

In addition to recombinant production methods, polypeptides of the invention, and fragments thereof, may be produced by direct peptide synthesis using solid-phase techniques (Merrifield J. (1963) *J. Am. Chem. Soc.* 85:2149-2154). Protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin

Elmer). Alternatively, various fragments may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

SITE-SPECIFIC MUTAGENESIS

Site-specific mutagenesis is a technique useful in the preparation of individual peptides, or biologically functional equivalent polypeptides, through specific mutagenesis of the underlying polynucleotides that encode them. The technique, well-known to those of skill in the art, further provides a ready ability to prepare and test sequence variants, for example, incorporating one or more of the foregoing considerations, by introducing one or more nucleotide sequence changes into the DNA. Site-specific mutagenesis allows the production of mutants through the use of specific oligonucleotide sequences which encode the DNA sequence of the desired mutation, as well as a sufficient number of adjacent nucleotides, to provide a primer sequence of sufficient size and sequence complexity to form a stable duplex on both sides of the deletion junction being traversed. Mutations may be employed in a selected polynucleotide sequence to improve, alter, decrease, modify, or otherwise change the properties of the polynucleotide itself, and/or alter the properties, activity, composition, stability, or primary sequence of the encoded polypeptide.

In certain embodiments of the present invention, the inventors contemplate the mutagenesis of the disclosed polynucleotide sequences to alter one or more properties of the encoded polypeptide, such as the antigenicity of a polypeptide vaccine. The techniques of site-specific mutagenesis are well-known in the art, and are widely used to create variants of both polypeptides and polynucleotides. For example, site-specific mutagenesis is often used to alter a specific portion of a DNA molecule. In such embodiments, a primer comprising typically about 14 to about 25 nucleotides or so in length is employed, with about 5 to about 10 residues on both sides of the junction of the sequence being altered.

As will be appreciated by those of skill in the art, site-specific mutagenesis techniques have often employed a phage vector that exists in both a single stranded and

double stranded form. Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage. These phage are readily commercially-available and their use is generally well-known to those skilled in the art. Double-stranded plasmids are also routinely employed in site directed mutagenesis that eliminates the step of transferring the gene of interest from a plasmid to a phage.

In general, site-directed mutagenesis in accordance herewith is performed by first obtaining a single-stranded vector or melting apart of two strands of a double-stranded vector that includes within its sequence a DNA sequence that encodes the desired peptide. An oligonucleotide primer bearing the desired mutated sequence is prepared, generally synthetically. This primer is then annealed with the single-stranded vector, and subjected to DNA polymerizing enzymes such as *E. coli* polymerase I Klenow fragment, in order to complete the synthesis of the mutation-bearing strand. Thus, a heteroduplex is formed wherein one strand encodes the original non-mutated sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform appropriate cells, such as *E. coli* cells, and clones are selected which include recombinant vectors bearing the mutated sequence arrangement.

The preparation of sequence variants of the selected peptide-encoding DNA segments using site-directed mutagenesis provides a means of producing potentially useful species and is not meant to be limiting as there are other ways in which sequence variants of peptides and the DNA sequences encoding them may be obtained. For example, recombinant vectors encoding the desired peptide sequence may be treated with mutagenic agents, such as hydroxylamine, to obtain sequence variants. Specific details regarding these methods and protocols are found in the teachings of Maloy *et al.*, 1994; Segal, 1976; Prokop and Bajpai, 1991; Kuby, 1994; and Maniatis *et al.*, 1982, each incorporated herein by reference, for that purpose.

As used herein, the term "oligonucleotide directed mutagenesis procedure" refers to template-dependent processes and vector-mediated propagation which result in an increase in the concentration of a specific nucleic acid molecule relative to its initial concentration, or in an increase in the concentration of a detectable signal, such as

amplification. As used herein, the term "oligonucleotide directed mutagenesis procedure" is intended to refer to a process that involves the template-dependent extension of a primer molecule. The term template dependent process refers to nucleic acid synthesis of an RNA or a DNA molecule wherein the sequence of the newly synthesized strand of nucleic acid is dictated by the well-known rules of complementary base pairing (see, for example, Watson, 1987). Typically, vector mediated methodologies involve the introduction of the nucleic acid fragment into a DNA or RNA vector, the clonal amplification of the vector, and the recovery of the amplified nucleic acid fragment. Examples of such methodologies are provided by U. S. Patent No. 4,237,224, specifically incorporated herein by reference in its entirety.

POLYNUCLEOTIDE AMPLIFICATION TECHNIQUES

A number of template dependent processes are available to amplify the target sequences of interest present in a sample. One of the best known amplification methods is the polymerase chain reaction (PCR™) which is described in detail in U.S. Patent Nos. 4,683,195, 4,683,202 and 4,800,159, each of which is incorporated herein by reference in its entirety. Briefly, in PCR™, two primer sequences are prepared which are complementary to regions on opposite complementary strands of the target sequence. An excess of deoxynucleoside triphosphates is added to a reaction mixture along with a DNA polymerase (*e.g.*, *Taq* polymerase). If the target sequence is present in a sample, the primers will bind to the target and the polymerase will cause the primers to be extended along the target sequence by adding on nucleotides. By raising and lowering the temperature of the reaction mixture, the extended primers will dissociate from the target to form reaction products, excess primers will bind to the target and to the reaction product and the process is repeated. Preferably reverse transcription and PCR™ amplification procedure may be performed in order to quantify the amount of mRNA amplified. Polymerase chain reaction methodologies are well known in the art.

Another method for amplification is the ligase chain reaction (referred to as LCR), disclosed in Eur. Pat. Appl. Publ. No. 320,308 (specifically incorporated herein by

reference in its entirety). In LCR, two complementary probe pairs are prepared, and in the presence of the target sequence, each pair will bind to opposite complementary strands of the target such that they abut. In the presence of a ligase, the two probe pairs will link to form a single unit. By temperature cycling, as in PCR™, bound ligated units dissociate from the target and then serve as "target sequences" for ligation of excess probe pairs. U.S. Patent No. 4,883,750, incorporated herein by reference in its entirety, describes an alternative method of amplification similar to LCR for binding probe pairs to a target sequence.

Qbeta Replicase, described in PCT Intl. Pat. Appl. Publ. No. PCT/US87/00880, incorporated herein by reference in its entirety, may also be used as still another amplification method in the present invention. In this method, a replicative sequence of RNA that has a region complementary to that of a target is added to a sample in the presence of an RNA polymerase. The polymerase will copy the replicative sequence that can then be detected.

An isothermal amplification method, in which restriction endonucleases and ligases are used to achieve the amplification of target molecules that contain nucleotide 5'-[α-thio]triphosphates in one strand of a restriction site (Walker *et al.*, 1992, incorporated herein by reference in its entirety), may also be useful in the amplification of nucleic acids in the present invention.

Strand Displacement Amplification (SDA) is another method of carrying out isothermal amplification of nucleic acids which involves multiple rounds of strand displacement and synthesis, *i.e.* nick translation. A similar method, called Repair Chain Reaction (RCR) is another method of amplification which may be useful in the present invention and is involves annealing several probes throughout a region targeted for amplification, followed by a repair reaction in which only two of the four bases are present. The other two bases can be added as biotinylated derivatives for easy detection. A similar approach is used in SDA.

Sequences can also be detected using a cyclic probe reaction (CPR). In CPR, a probe having a 3' and 5' sequences of non-target DNA and an internal or "middle"

sequence of the target protein specific RNA is hybridized to DNA which is present in a sample. Upon hybridization, the reaction is treated with RNaseH, and the products of the probe are identified as distinctive products by generating a signal that is released after digestion. The original template is annealed to another cycling probe and the reaction is
5 repeated. Thus, CPR involves amplifying a signal generated by hybridization of a probe to a target gene specific expressed nucleic acid.

Still other amplification methods described in Great Britain Pat. Appl. No. 2 202 328, and in PCT Intl. Pat. Appl. Publ. No. PCT/US89/01025, each of which is incorporated herein by reference in its entirety, may be used in accordance with the present
10 invention. In the former application, "modified" primers are used in a PCR-like, template and enzyme dependent synthesis. The primers may be modified by labeling with a capture moiety (*e.g.*, biotin) and/or a detector moiety (*e.g.*, enzyme). In the latter application, an excess of labeled probes is added to a sample. In the presence of the target sequence, the probe binds and is cleaved catalytically. After cleavage, the target sequence is released
15 intact to be bound by excess probe. Cleavage of the labeled probe signals the presence of the target sequence.

Other nucleic acid amplification procedures include transcription-based amplification systems (TAS) (Kwoh *et al.*, 1989; PCT Intl. Pat. Appl. Publ. No. WO 88/10315, incorporated herein by reference in its entirety), including nucleic acid sequence
20 based amplification (NASBA) and 3SR. In NASBA, the nucleic acids can be prepared for amplification by standard phenol/chloroform extraction, heat denaturation of a sample, treatment with lysis buffer and minispin columns for isolation of DNA and RNA or guanidinium chloride extraction of RNA. These amplification techniques involve annealing a primer that has sequences specific to the target sequence. Following
25 polymerization, DNA/RNA hybrids are digested with RNase H while double stranded DNA molecules are heat-denatured again. In either case the single stranded DNA is made fully double stranded by addition of second target-specific primer, followed by polymerization. The double stranded DNA molecules are then multiply transcribed by a polymerase such as T7 or SP6. In an isothermal cyclic reaction, the RNAs are reverse

transcribed into DNA, and transcribed once again with a polymerase such as T7 or SP6. The resulting products, whether truncated or complete, indicate target-specific sequences.

Eur. Pat. Appl. Publ. No. 329,822, incorporated herein by reference in its entirety, disclose a nucleic acid amplification process involving cyclically synthesizing
 5 single-stranded RNA ("ssRNA"), ssDNA, and double-stranded DNA (dsDNA), which may be used in accordance with the present invention. The ssRNA is a first template for a first primer oligonucleotide, which is elongated by reverse transcriptase (RNA-dependent DNA polymerase). The RNA is then removed from resulting DNA:RNA duplex by the action of
 10 ribonuclease H (RNase H, an RNase specific for RNA in a duplex with either DNA or RNA). The resultant ssDNA is a second template for a second primer, which also includes the sequences of an RNA polymerase promoter (exemplified by T7 RNA polymerase) 5' to its homology to its template. This primer is then extended by DNA polymerase (exemplified by the large "Klenow" fragment of *E. coli* DNA polymerase I), resulting as a
 15 double-stranded DNA ("dsDNA") molecule, having a sequence identical to that of the original RNA between the primers and having additionally, at one end, a promoter sequence. This promoter sequence can be used by the appropriate RNA polymerase to make many RNA copies of the DNA. These copies can then re-enter the cycle leading to very swift amplification. With proper choice of enzymes, this amplification can be done
 20 isothermally without addition of enzymes at each cycle. Because of the cyclical nature of this process, the starting sequence can be chosen to be in the form of either DNA or RNA.

PCT Intl. Pat. Appl. Publ. No. WO 89/06700, incorporated herein by reference in its entirety, disclose a nucleic acid sequence amplification scheme based on the hybridization of a promoter/primer sequence to a target single-stranded DNA ("ssDNA") followed by transcription of many RNA copies of the sequence. This scheme is not cyclic;
 25 *i.e.* new templates are not produced from the resultant RNA transcripts. Other amplification methods include "RACE" (Frohman, 1990), and "one-sided PCR" (Ohara, 1989) which are well-known to those of skill in the art.

Methods based on ligation of two (or more) oligonucleotides in the presence of nucleic acid having the sequence of the resulting "di-oligonucleotide", thereby

amplifying the di-oligonucleotide (Wu and Dean, 1996, incorporated herein by reference in its entirety), may also be used in the amplification of DNA sequences of the present invention.

BIOLOGICAL FUNCTIONAL EQUIVALENTS

5 Modification and changes may be made in the structure of the polynucleotides and polypeptides of the present invention and still obtain a functional molecule that encodes a polypeptide with desirable characteristics. As mentioned above, it is often desirable to introduce one or more mutations into a specific polynucleotide sequence. In certain circumstances, the resulting encoded polypeptide sequence is altered
10 by this mutation, or in other cases, the sequence of the polypeptide is unchanged by one or more mutations in the encoding polynucleotide.

When it is desirable to alter the amino acid sequence of a polypeptide to create an equivalent, or even an improved, second-generation molecule, the amino acid changes may be achieved by changing one or more of the codons of the encoding DNA
15 sequence, according to Table 1.

For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a protein that defines
20 that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence, and, of course, its underlying DNA coding sequence, and nevertheless obtain a protein with like properties. It is thus contemplated by the inventors that various changes may be made in the peptide sequences of the disclosed compositions, or corresponding DNA sequences which encode said peptides without appreciable loss of
25 their biological utility or activity.

TABLE 1

Amino Acids			Codons						
Alanine	Ala	A	GCA	GCC	GCG	GCU			
Cysteine	Cys	C	UGC	UGU					
Aspartic acid	Asp	D	GAC	GAU					
Glutamic acid	Glu	E	GAA	GAG					
Phenylalanine	Phe	F	UUC	UUU					
Glycine	Gly	G	GGA	GGC	GGG	GGU			
Histidine	His	H	CAC	CAU					
Isoleucine	Ile	I	AUA	AUC	AUU				
Lysine	Lys	K	AAA	AAG					
Leucine	Leu	L	UUA	UUG	CUA	CUC	CUG	CUU	
Methionine	Met	M	AUG						
Asparagine	Asn	N	AAC	AAU					
Proline	Pro	P	CCA	CCC	CCG	CCU			
Glutamine	Gln	Q	CAA	CAG					
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGU	
Serine	Ser	S	AGC	AGU	UCA	UCC	UCG	UCU	
Threonine	Thr	T	ACA	ACC	ACG	ACU			
Valine	Val	V	GUA	GUC	GUG	GUU			
Tryptophan	Trp	W	UGG						
Tyrosine	Tyr	Y	UAC	UAU					

In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, 1982, incorporated herein by reference). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes,

substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics (Kyte and Doolittle, 1982). These values are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8);
 5 glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a protein with
 10 similar biological activity, *i.e.* still obtain a biological functionally equivalent protein. In making such changes, the substitution of amino acids whose hydropathic indices are within ± 2 is preferred, those within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred. It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U. S. Patent 4,554,101
 15 (specifically incorporated herein by reference in its entirety), states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein.

As detailed in U. S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 \pm 1);
 20 1); glutamate (+3.0 \pm 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 \pm 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically
 25 equivalent, and in particular, an immunologically equivalent protein. In such changes, the substitution of amino acids whose hydrophilicity values are within ± 2 is preferred, those within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

In addition, any polynucleotide may be further modified to increase stability *in vivo*. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages in the backbone; and/or the inclusion of nontraditional bases such as inosine, queosine and wybutosine, as well as acetyl- methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine and uridine.

IN VIVO POLYNUCLEOTIDE DELIVERY TECHNIQUES

In additional embodiments, genetic constructs comprising one or more of the polynucleotides of the invention are introduced into cells *in vivo*. This may be achieved using any of a variety of well known approaches, several of which are outlined below for the purpose of illustration.

1. ADENOVIRUS

One of the preferred methods for *in vivo* delivery of one or more nucleic acid sequences involves the use of an adenovirus expression vector. "Adenovirus expression vector" is meant to include those constructs containing adenovirus sequences sufficient to (a) support packaging of the construct and (b) to express a polynucleotide that has been cloned therein in a sense or antisense orientation. Of course, in the context of an antisense construct, expression does not require that the gene product be synthesized.

The expression vector comprises a genetically engineered form of an adenovirus. Knowledge of the genetic organization of adenovirus, a 36 kb, linear, double-stranded DNA virus, allows substitution of large pieces of adenoviral DNA with foreign

sequences up to 7 kb (Grunhaus and Horwitz, 1992). In contrast to retrovirus, the adenoviral infection of host cells does not result in chromosomal integration because adenoviral DNA can replicate in an episomal manner without potential genotoxicity. Also, adenoviruses are structurally stable, and no genome rearrangement has been detected after
5 extensive amplification. Adenovirus can infect virtually all epithelial cells regardless of their cell cycle stage. So far, adenoviral infection appears to be linked only to mild disease such as acute respiratory disease in humans.

Adenovirus is particularly suitable for use as a gene transfer vector because of its mid-sized genome, ease of manipulation, high titer, wide target-cell range and high
10 infectivity. Both ends of the viral genome contain 100-200 base pair inverted repeats (ITRs), which are *cis* elements necessary for viral DNA replication and packaging. The early (E) and late (L) regions of the genome contain different transcription units that are divided by the onset of viral DNA replication. The E1 region (E1A and E1B) encodes proteins responsible for the regulation of transcription of the viral genome and a few
15 cellular genes. The expression of the E2 region (E2A and E2B) results in the synthesis of the proteins for viral DNA replication. These proteins are involved in DNA replication, late gene expression and host cell shut-off (Renan, 1990). The products of the late genes, including the majority of the viral capsid proteins, are expressed only after significant processing of a single primary transcript issued by the major late promoter (MLP). The
20 MLP, (located at 16.8 m.u.) is particularly efficient during the late phase of infection, and all the mRNA's issued from this promoter possess a 5'-tripartite leader (TPL) sequence which makes them preferred mRNA's for translation.

In a current system, recombinant adenovirus is generated from homologous recombination between shuttle vector and provirus vector. Due to the possible
25 recombination between two proviral vectors, wild-type adenovirus may be generated from this process. Therefore, it is critical to isolate a single clone of virus from an individual plaque and examine its genomic structure.

Generation and propagation of the current adenovirus vectors, which are replication deficient, depend on a unique helper cell line, designated 293, which was

transformed from human embryonic kidney cells by Ad5 DNA fragments and constitutively expresses E1 proteins (Graham *et al.*, 1977). Since the E3 region is dispensable from the adenovirus genome (Jones and Shenk, 1978), the current adenovirus vectors, with the help of 293 cells, carry foreign DNA in either the E1, the D3 or both regions (Graham and Prevec, 1991). In nature, adenovirus can package approximately 105% of the wild-type genome (Ghosh-Choudhury *et al.*, 1987), providing capacity for about 2 extra kB of DNA. Combined with the approximately 5.5 kB of DNA that is replaceable in the E1 and E3 regions, the maximum capacity of the current adenovirus vector is under 7.5 kB, or about 15% of the total length of the vector. More than 80% of the adenovirus viral genome remains in the vector backbone and is the source of vector-borne cytotoxicity. Also, the replication deficiency of the E1-deleted virus is incomplete. For example, leakage of viral gene expression has been observed with the currently available vectors at high multiplicities of infection (MOI) (Mulligan, 1993).

Helper cell lines may be derived from human cells such as human embryonic kidney cells, muscle cells, hematopoietic cells or other human embryonic mesenchymal or epithelial cells. Alternatively, the helper cells may be derived from the cells of other mammalian species that are permissive for human adenovirus. Such cells include, *e.g.*, Vero cells or other monkey embryonic mesenchymal or epithelial cells. As stated above, the currently preferred helper cell line is 293.

Recently, Racher *et al.* (1995) disclosed improved methods for culturing 293 cells and propagating adenovirus. In one format, natural cell aggregates are grown by inoculating individual cells into 1 liter siliconized spinner flasks (Techne, Cambridge, UK) containing 100-200 ml of medium. Following stirring at 40 rpm, the cell viability is estimated with trypan blue. In another format, Fibra-Cel microcarriers (Bibby Sterlin, Stone, UK) (5 g/l) is employed as follows. A cell inoculum, resuspended in 5 ml of medium, is added to the carrier (50 ml) in a 250 ml Erlenmeyer flask and left stationary, with occasional agitation, for 1 to 4 h. The medium is then replaced with 50 ml of fresh medium and shaking initiated. For virus production, cells are allowed to grow to about 80% confluence, after which time the medium is replaced (to 25% of the final volume) and

adenovirus added at an MOI of 0.05. Cultures are left stationary overnight, following which the volume is increased to 100% and shaking commenced for another 72 h.

Other than the requirement that the adenovirus vector be replication defective, or at least conditionally defective, the nature of the adenovirus vector is not believed to be crucial to the successful practice of the invention. The adenovirus may be of any of the 42 different known serotypes or subgroups A-F. Adenovirus type 5 of subgroup C is the preferred starting material in order to obtain a conditional replication-defective adenovirus vector for use in the present invention, since Adenovirus type 5 is a human adenovirus about which a great deal of biochemical and genetic information is known, and it has historically been used for most constructions employing adenovirus as a vector.

As stated above, the typical vector according to the present invention is replication defective and will not have an adenovirus E1 region. Thus, it will be most convenient to introduce the polynucleotide encoding the gene of interest at the position from which the E1-coding sequences have been removed. However, the position of insertion of the construct within the adenovirus sequences is not critical to the invention. The polynucleotide encoding the gene of interest may also be inserted in lieu of the deleted E3 region in E3 replacement vectors as described by Karlsson *et al.* (1986) or in the E4 region where a helper cell line or helper virus complements the E4 defect.

Adenovirus is easy to grow and manipulate and exhibits broad host range *in vitro* and *in vivo*. This group of viruses can be obtained in high titers, *e.g.*, 10^9 - 10^{11} plaque-forming units per ml, and they are highly infective. The life cycle of adenovirus does not require integration into the host cell genome. The foreign genes delivered by adenovirus vectors are episomal and, therefore, have low genotoxicity to host cells. No side effects have been reported in studies of vaccination with wild-type adenovirus (Couch *et al.*, 1963; Top *et al.*, 1971), demonstrating their safety and therapeutic potential as *in vivo* gene transfer vectors.

Adenovirus vectors have been used in eukaryotic gene expression (Levrero *et al.*, 1991; Gomez-Foix *et al.*, 1992) and vaccine development (Grunhaus and Horwitz, 1992; Graham and Prevec, 1992). Recently, animal studies suggested that recombinant

adenovirus could be used for gene therapy (Stratford-Perricaudet and Perricaudet, 1991; Stratford-Perricaudet *et al.*, 1990; Rich *et al.*, 1993). Studies in administering recombinant adenovirus to different tissues include trachea instillation (Rosenfeld *et al.*, 1991; Rosenfeld *et al.*, 1992), muscle injection (Ragot *et al.*, 1993), peripheral intravenous
 5 injections (Herz and Gerard, 1993) and stereotactic inoculation into the brain (Le Gal La Salle *et al.*, 1993).

2. RETROVIRUSES

The retroviruses are a group of single-stranded RNA viruses characterized by an ability to convert their RNA to double-stranded DNA in infected cells by a process of
 10 reverse-transcription (Coffin, 1990). The resulting DNA then stably integrates into cellular chromosomes as a provirus and directs synthesis of viral proteins. The integration results in the retention of the viral gene sequences in the recipient cell and its descendants. The retroviral genome contains three genes, gag, pol, and env that code for capsid proteins, polymerase enzyme, and envelope components, respectively. A sequence found upstream
 15 from the gag gene contains a signal for packaging of the genome into virions. Two long terminal repeat (LTR) sequences are present at the 5' and 3' ends of the viral genome. These contain strong promoter and enhancer sequences and are also required for integration in the host cell genome (Coffin, 1990).

In order to construct a retroviral vector, a nucleic acid encoding one or more
 20 oligonucleotide or polynucleotide sequences of interest is inserted into the viral genome in the place of certain viral sequences to produce a virus that is replication-defective. In order to produce virions, a packaging cell line containing the gag, pol, and env genes but without the LTR and packaging components is constructed (Mann *et al.*, 1983). When a recombinant plasmid containing a cDNA, together with the retroviral LTR and packaging
 25 sequences is introduced into this cell line (by calcium phosphate precipitation for example), the packaging sequence allows the RNA transcript of the recombinant plasmid to be packaged into viral particles, which are then secreted into the culture media (Nicolas and Rubenstein, 1988; Temin, 1986; Mann *et al.*, 1983). The media containing the

recombinant retroviruses is then collected, optionally concentrated, and used for gene transfer. Retroviral vectors are able to infect a broad variety of cell types. However, integration and stable expression require the division of host cells (Paskind *et al.*, 1975).

5 A novel approach designed to allow specific targeting of retrovirus vectors was recently developed based on the chemical modification of a retrovirus by the chemical addition of lactose residues to the viral envelope. This modification could permit the specific infection of hepatocytes *via* sialoglycoprotein receptors.

10 A different approach to targeting of recombinant retroviruses was designed in which biotinylated antibodies against a retroviral envelope protein and against a specific cell receptor were used. The antibodies were coupled *via* the biotin components by using streptavidin (Roux *et al.*, 1989). Using antibodies against major histocompatibility complex class I and class II antigens, they demonstrated the infection of a variety of human cells that bore those surface antigens with an ecotropic virus *in vitro* (Roux *et al.*, 1989).

3. ADENO-ASSOCIATED VIRUSES

15 AAV (Ridgeway, 1988; Hermonat and Muzyczka, 1984) is a parovirus, discovered as a contamination of adenoviral stocks. It is a ubiquitous virus (antibodies are present in 85% of the US human population) that has not been linked to any disease. It is also classified as a dependovirus, because its replications is dependent on the presence of a helper virus, such as adenovirus. Five serotypes have been isolated, of which AAV-2 is the
20 best characterized. AAV has a single-stranded linear DNA that is encapsidated into capsid proteins VP1, VP2 and VP3 to form an icosahedral virion of 20 to 24 nm in diameter (Muzyczka and McLaughlin, 1988).

The AAV DNA is approximately 4.7 kilobases long. It contains two open reading frames and is flanked by two ITRs. There are two major genes in the AAV
25 genome: *rep* and *cap*. The *rep* gene codes for proteins responsible for viral replications, whereas *cap* codes for capsid protein VP1-3. Each ITR forms a T-shaped hairpin structure. These terminal repeats are the only essential *cis* components of the AAV for chromosomal integration. Therefore, the AAV can be used as a vector with all viral coding

There are several factors that prompted researchers to study the possibility of using rAAV as an expression vector. One is that the requirements for delivering a gene to integrate into the host chromosome are surprisingly few. It is necessary to have the 145-bp ITRs, which are only 6% of the AAV genome. This leaves room in the vector to assemble a 4.5-kb DNA insertion. While this carrying capacity may prevent the AAV from delivering large genes, it is amply suited for delivering the antisense constructs of the present invention.

AAV is also a good choice of delivery vehicles due to its safety. There is a relatively complicated rescue mechanism: not only wild type adenovirus but also AAV genes are required to mobilize rAAV. Likewise, AAV is not pathogenic and not associated with any disease. The removal of viral coding sequences minimizes immune reactions to viral gene expression, and therefore, rAAV does not evoke an inflammatory response.

Other viral vectors may be employed as expression constructs in the present invention for the delivery of oligonucleotide or polynucleotide sequences to a host cell. Vectors derived from viruses such as vaccinia virus (Ridgeway, 1988; Coupar *et al.*, 1988), lentiviruses, polio viruses and herpes viruses may be employed. They offer several attractive features for various mammalian cells (Friedmann, 1989; Ridgeway, 1988; Coupar *et al.*, 1988; Horwich *et al.*, 1990).

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material. The hepatotropism and persistence (integration) were particularly attractive properties for liver-directed gene transfer. Chang *et al.* (1991) introduced the chloramphenicol acetyltransferase (CAT) gene into duck hepatitis B virus genome in the place of the polymerase, surface, and pre-surface coding sequences. It was cotransfected with wild-type virus into an avian hepatoma cell line. Culture media containing high titers of the recombinant virus were used to infect primary duckling hepatocytes. Stable CAT gene expression was detected for at least 24 days after transfection (Chang *et al.*, 1991).

5. NON-VIRAL VECTORS

In order to effect expression of the oligonucleotide or polynucleotide sequences of the present invention, the expression construct must be delivered into a cell. This delivery may be accomplished *in vitro*, as in laboratory procedures for transforming cells lines, or *in vivo* or *ex vivo*, as in the treatment of certain disease states. As described above, one preferred mechanism for delivery is *via* viral infection where the expression construct is encapsulated in an infectious viral particle.

Once the expression construct has been delivered into the cell the nucleic acid encoding the desired oligonucleotide or polynucleotide sequences may be positioned and expressed at different sites. In certain embodiments, the nucleic acid encoding the construct may be stably integrated into the genome of the cell. This integration may be in the specific location and orientation *via* homologous recombination (gene replacement) or it may be integrated in a random, non-specific location (gene augmentation). In yet further embodiments, the nucleic acid may be stably maintained in the cell as a separate, episomal segment of DNA. Such nucleic acid segments or "episomes" encode sequences sufficient to permit maintenance and replication independent of or in synchronization with the host cell cycle. How the expression construct is delivered to a cell and where in the cell the nucleic acid remains is dependent on the type of expression construct employed.

In certain embodiments of the invention, the expression construct comprising one or more oligonucleotide or polynucleotide sequences may simply consist of naked recombinant DNA or plasmids. Transfer of the construct may be performed by any

of the methods mentioned above which physically or chemically permeabilize the cell membrane. This is particularly applicable for transfer *in vitro* but it may be applied to *in vivo* use as well. Dubensky *et al.* (1984) successfully injected polyomavirus DNA in the form of calcium phosphate precipitates into liver and spleen of adult and newborn mice demonstrating active viral replication and acute infection. Benvenisty and Reshef (1986) also demonstrated that direct intraperitoneal injection of calcium phosphate-precipitated plasmids results in expression of the transfected genes. It is envisioned that DNA encoding a gene of interest may also be transferred in a similar manner *in vivo* and express the gene product.

Another embodiment of the invention for transferring a naked DNA expression construct into cells may involve particle bombardment. This method depends on the ability to accelerate DNA-coated microprojectiles to a high velocity allowing them to pierce cell membranes and enter cells without killing them (Klein *et al.*, 1987). Several devices for accelerating small particles have been developed. One such device relies on a high voltage discharge to generate an electrical current, which in turn provides the motive force (Yang *et al.*, 1990). The microprojectiles used have consisted of biologically inert substances such as tungsten or gold beads.

Selected organs including the liver, skin, and muscle tissue of rats and mice have been bombarded *in vivo* (Yang *et al.*, 1990; Zelenin *et al.*, 1991). This may require surgical exposure of the tissue or cells, to eliminate any intervening tissue between the gun and the target organ, *i.e.* *ex vivo* treatment. Again, DNA encoding a particular gene may be delivered *via* this method and still be incorporated by the present invention.

ANTISENSE OLIGONUCLEOTIDES

The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and

an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, synthetic antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

5 The targeting of antisense oligonucleotides to mRNA is thus one mechanism to shut down protein synthesis, and, consequently, represents a powerful and targeted therapeutic approach. For example, the synthesis of polygalacturonase and the muscarine type 2 acetylcholine receptor are inhibited by antisense oligonucleotides directed to their respective mRNA sequences (U. S. Patent 5,739,119 and U. S. Patent 5,759,829, each
10 specifically incorporated herein by reference in its entirety). Further, examples of antisense inhibition have been demonstrated with the nuclear protein cyclin, the multiple drug resistance gene (MDG1), ICAM-1, E-selectin, STK-1, striatal GABA_A receptor and human EGF (Jaskulski *et al.*, 1988; Vasanthakumar and Ahmed, 1989; Peris *et al.*, 1998; U. S. Patent 5,801,154; U. S. Patent 5,789,573; U. S. Patent 5,718,709 and U. S. Patent
15 5,610,288, each specifically incorporated herein by reference in its entirety). Antisense constructs have also been described that inhibit and can be used to treat a variety of abnormal cellular proliferations, *e.g.* cancer (U. S. Patent 5,747,470; U. S. Patent 5,591,317 and U. S. Patent 5,783,683, each specifically incorporated herein by reference in its entirety).

20 Therefore, in exemplary embodiments, the invention provides oligonucleotide sequences that comprise all, or a portion of, any sequence that is capable of specifically binding to polynucleotide sequence described herein, or a complement thereof. In one embodiment, the antisense oligonucleotides comprise DNA or derivatives thereof. In another embodiment, the oligonucleotides comprise RNA or derivatives thereof. In a
25 third embodiment, the oligonucleotides are modified DNAs comprising a phosphorothioated modified backbone. In a fourth embodiment, the oligonucleotide sequences comprise peptide nucleic acids or derivatives thereof. In each case, preferred compositions comprise a sequence region that is complementary, and more preferably

substantially-complementary, and even more preferably, completely complementary to one or more portions of polynucleotides disclosed herein.

Selection of antisense compositions specific for a given gene sequence is based upon analysis of the chosen target sequence (*i.e.* in these illustrative examples the rat and human sequences) and determination of secondary structure, T_m , binding energy, relative stability, and antisense compositions were selected based upon their relative inability to form dimers, hairpins, or other secondary structures that would reduce or prohibit specific binding to the target mRNA in a host cell.

Highly preferred target regions of the mRNA, are those which are at or near the AUG translation initiation codon, and those sequences which were substantially complementary to 5' regions of the mRNA. These secondary structure analyses and target site selection considerations were performed using v.4 of the OLIGO primer analysis software (Rychlik, 1997) and the BLASTN 2.0.5 algorithm software (Altschul *et al.*, 1997).

The use of an antisense delivery method employing a short peptide vector, termed MPG (27 residues), is also contemplated. The MPG peptide contains a hydrophobic domain derived from the fusion sequence of HIV gp41 and a hydrophilic domain from the nuclear localization sequence of SV40 T-antigen (Morris *et al.*, 1997). It has been demonstrated that several molecules of the MPG peptide coat the antisense oligonucleotides and can be delivered into cultured mammalian cells in less than 1 hour with relatively high efficiency (90%). Further, the interaction with MPG strongly increases both the stability of the oligonucleotide to nuclease and the ability to cross the plasma membrane (Morris *et al.*, 1997).

RIBOZYMES

Although proteins traditionally have been used for catalysis of nucleic acids, another class of macromolecules has emerged as useful in this endeavor. Ribozymes are RNA-protein complexes that cleave nucleic acids in a site-specific fashion. Ribozymes have specific catalytic domains that possess endonuclease activity (Kim and Cech, 1987; Gerlach *et al.*, 1987; Forster and Symons, 1987). For example, a large number of

ribozymes accelerate phosphoester transfer reactions with a high degree of specificity, often cleaving only one of several phosphoesters in an oligonucleotide substrate (Cech *et al.*, 1981; Michel and Westhof, 1990; Reinhold-Hurek and Shub, 1992). This specificity has been attributed to the requirement that the substrate bind via specific base-pairing
5 interactions to the internal guide sequence ("IGS") of the ribozyme prior to chemical reaction.

Ribozyme catalysis has primarily been observed as part of sequence-specific cleavage/ligation reactions involving nucleic acids (Joyce, 1989; Cech *et al.*, 1981). For example, U. S. Patent No. 5,354,855 (specifically incorporated herein by reference) reports
10 that certain ribozymes can act as endonucleases with a sequence specificity greater than that of known ribonucleases and approaching that of the DNA restriction enzymes. Thus, sequence-specific ribozyme-mediated inhibition of gene expression may be particularly suited to therapeutic applications (Scanlon *et al.*, 1991; Sarver *et al.*, 1990). Recently, it was reported that ribozymes elicited genetic changes in some cells lines to which they were
15 applied; the altered genes included the oncogenes *H-ras*, *c-fos* and genes of HIV. Most of this work involved the modification of a target mRNA, based on a specific mutant codon that is cleaved by a specific ribozyme.

Six basic varieties of naturally-occurring enzymatic RNAs are known presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds *in trans* (and
20 thus can cleave other RNA molecules) under physiological conditions. In general, enzymatic nucleic acids act by first binding to a target RNA. Such binding occurs through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base-
25 pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

The enzymatic nature of a ribozyme is advantageous over many technologies, such as antisense technology (where a nucleic acid molecule simply binds to a nucleic acid target to block its translation) since the concentration of ribozyme necessary to affect a therapeutic treatment is lower than that of an antisense oligonucleotide. This advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly specific inhibitor, with the specificity of inhibition depending not only on the base pairing mechanism of binding to the target RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or base-substitutions, near the site of cleavage can completely eliminate catalytic activity of a ribozyme. Similar mismatches in antisense molecules do not prevent their action (Woolf *et al.*, 1992). Thus, the specificity of action of a ribozyme is greater than that of an antisense oligonucleotide binding the same RNA site.

The enzymatic nucleic acid molecule may be formed in a hammerhead, hairpin, a hepatitis δ virus, group I intron or RNaseP RNA (in association with an RNA guide sequence) or Neurospora VS RNA motif. Examples of hammerhead motifs are described by Rossi *et al.* (1992). Examples of hairpin motifs are described by Hampel *et al.* (Eur. Pat. Appl. Publ. No. EP 0360257), Hampel and Tritz (1989), Hampel *et al.* (1990) and U. S. Patent 5,631,359 (specifically incorporated herein by reference). An example of the hepatitis δ virus motif is described by Perrotta and Been (1992); an example of the RNaseP motif is described by Guerrier-Takada *et al.* (1983); Neurospora VS RNA ribozyme motif is described by Collins (Saville and Collins, 1990; Saville and Collins, 1991; Collins and Olive, 1993); and an example of the Group I intron is described in (U. S. Patent 4,987,071, specifically incorporated herein by reference). All that is important in an enzymatic nucleic acid molecule of this invention is that it has a specific substrate binding site which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule. Thus the ribozyme constructs need not be limited to specific motifs mentioned herein.

In certain embodiments, it may be important to produce enzymatic cleaving agents which exhibit a high degree of specificity for the RNA of a desired target, such as one of the sequences disclosed herein. The enzymatic nucleic acid molecule is preferably targeted to a highly conserved sequence region of a target mRNA. Such enzymatic nucleic acid molecules can be delivered exogenously to specific cells as required. Alternatively, the ribozymes can be expressed from DNA or RNA vectors that are delivered to specific cells.

Small enzymatic nucleic acid motifs (e.g., of the hammerhead or the hairpin structure) may also be used for exogenous delivery. The simple structure of these molecules increases the ability of the enzymatic nucleic acid to invade targeted regions of the mRNA structure. Alternatively, catalytic RNA molecules can be expressed within cells from eukaryotic promoters (e.g., Scanlon *et al.*, 1991; Kashani-Sabet *et al.*, 1992; Dropulic *et al.*, 1992; Weerasinghe *et al.*, 1991; Ojwang *et al.*, 1992; Chen *et al.*, 1992; Sarver *et al.*, 1990). Those skilled in the art realize that any ribozyme can be expressed in eukaryotic cells from the appropriate DNA vector. The activity of such ribozymes can be augmented by their release from the primary transcript by a second ribozyme (Int. Pat. Appl. Publ. No. WO 93/23569, and Int. Pat. Appl. Publ. No. WO 94/02595, both hereby incorporated by reference; Ohkawa *et al.*, 1992; Taira *et al.*, 1991; and Ventura *et al.*, 1993).

Ribozymes may be added directly, or can be complexed with cationic lipids, lipid complexes, packaged within liposomes, or otherwise delivered to target cells. The RNA or RNA complexes can be locally administered to relevant tissues *ex vivo*, or *in vivo* through injection, aerosol inhalation, infusion pump or stent, with or without their incorporation in biopolymers.

Ribozymes may be designed as described in Int. Pat. Appl. Publ. No. WO 93/23569 and Int. Pat. Appl. Publ. No. WO 94/02595, each specifically incorporated herein by reference) and synthesized to be tested *in vitro* and *in vivo*, as described. Such ribozymes can also be optimized for delivery. While specific examples are provided, those in the art will recognize that equivalent RNA targets in other species can be utilized when necessary.

Hammerhead or hairpin ribozymes may be individually analyzed by computer folding (Jaeger *et al.*, 1989) to assess whether the ribozyme sequences fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core are eliminated from
 5 consideration. Varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 or so bases on each arm are able to bind to, or otherwise interact with, the target RNA.

Ribozymes of the hammerhead or hairpin motif may be designed to anneal to various sites in the mRNA message, and can be chemically synthesized. The method of
 10 synthesis used follows the procedure for normal RNA synthesis as described in Usman *et al.* (1987) and in Scaringe *et al.* (1990) and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. Average stepwise coupling yields are typically >98%. Hairpin ribozymes may be synthesized in two parts and annealed to reconstruct an active
 15 ribozyme (Chowrira and Burke, 1992). Ribozymes may be modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-fluoro, 2'-O-methyl, 2'-H (for a review see *e.g.*, Usman and Cedergren, 1992). Ribozymes may be purified by gel electrophoresis using general methods or by high pressure liquid chromatography and resuspended in water.

20 Ribozyme activity can be optimized by altering the length of the ribozyme binding arms, or chemically synthesizing ribozymes with modifications that prevent their degradation by serum ribonucleases (see *e.g.*, Int. Pat. Appl. Publ. No. WO 92/07065; Perrault *et al.*, 1990; Pieken *et al.*, 1991; Usman and Cedergren, 1992; Int. Pat. Appl. Publ. No. WO 93/15187; Int. Pat. Appl. Publ. No. WO 91/03162; Eur. Pat. Appl. Publ.
 25 No. 92110298.4; U. S. Patent 5,334,711; and Int. Pat. Appl. Publ. No. WO 94/13688, which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules), modifications which enhance their efficacy in cells, and removal of stem II bases to shorten RNA synthesis times and reduce chemical requirements.

Sullivan *et al.* (Int. Pat. Appl. Publ. No. WO 94/02595) describes the general methods for delivery of enzymatic RNA molecules. Ribozymes may be administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into
5 other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres. For some indications, ribozymes may be directly delivered *ex vivo* to cells or tissues with or without the aforementioned vehicles. Alternatively, the RNA/vehicle combination may be locally delivered by direct inhalation, by direct injection or by use of a catheter, infusion pump or stent. Other routes of delivery include, but are not
10 limited to, intravascular, intramuscular, subcutaneous or joint injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions of ribozyme delivery and administration are provided in Int. Pat. Appl. Publ. No. WO 94/02595 and Int. Pat. Appl. Publ. No. WO 93/23569, each specifically incorporated herein by reference.

15 Another means of accumulating high concentrations of a ribozyme(s) within cells is to incorporate the ribozyme-encoding sequences into a DNA expression vector. Transcription of the ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the
20 levels of a given pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, silencers, *etc.*) present nearby. Prokaryotic RNA polymerase promoters may also be used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990; Gao and Huang, 1993; Lieber *et al.*, 1993; Zhou *et al.*, 1990). Ribozymes expressed from such promoters
25 can function in mammalian cells (*e.g.* Kashani-Saber *et al.*, 1992; Ojwang *et al.*, 1992; Chen *et al.*, 1992; Yu *et al.*, 1993; L'Huillier *et al.*, 1992; Lisiewicz *et al.*, 1993). Such transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors

(such as adenovirus or adeno-associated vectors), or viral RNA vectors (such as retroviral, semliki forest virus, sindbis virus vectors).

Ribozymes may be used as diagnostic tools to examine genetic drift and mutations within diseased cells. They can also be used to assess levels of the target RNA molecule. The close relationship between ribozyme activity and the structure of the target RNA allows the detection of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple ribozymes, one may map nucleotide changes which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with ribozymes may be used to inhibit gene expression and define the role (essentially) of specified gene products in the progression of disease. In this manner, other genetic targets may be defined as important mediators of the disease. These studies will lead to better treatment of the disease progression by affording the possibility of combinational therapies (*e.g.*, multiple ribozymes targeted to different genes, ribozymes coupled with known small molecule inhibitors, or intermittent treatment with combinations of ribozymes and/or other chemical or biological molecules). Other *in vitro* uses of ribozymes are well known in the art, and include detection of the presence of mRNA associated with an IL-5 related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a ribozyme using standard methodology.

20 PEPTIDE NUCLEIC ACIDS

In certain embodiments, the inventors contemplate the use of peptide nucleic acids (PNAs) in the practice of the methods of the invention. PNA is a DNA mimic in which the nucleobases are attached to a pseudopeptide backbone (Good and Nielsen, 1997). PNA is able to be utilized in a number methods that traditionally have used RNA or DNA. Often PNA sequences perform better in techniques than the corresponding RNA or DNA sequences and have utilities that are not inherent to RNA or DNA. A review of PNA including methods of making, characteristics of, and methods of using, is provided by Corey (1997) and is incorporated herein by reference. As such, in certain embodiments,

one may prepare PNA sequences that are complementary to one or more portions of the ACE mRNA sequence, and such PNA compositions may be used to regulate, alter, decrease, or reduce the translation of ACE-specific mRNA, and thereby alter the level of ACE activity in a host cell to which such PNA compositions have been administered.

5 PNAs have 2-aminoethyl-glycine linkages replacing the normal phosphodiester backbone of DNA (Nielsen *et al.*, 1991; Hanvey *et al.*, 1992; Hyrup and Nielsen, 1996; Neilsen, 1996). This chemistry has three important consequences: firstly, in contrast to DNA or phosphorothioate oligonucleotides, PNAs are neutral molecules; secondly, PNAs are achiral, which avoids the need to develop a stereoselective synthesis; 10 and thirdly, PNA synthesis uses standard Boc (Dueholm *et al.*, 1994) or Fmoc (Thomson *et al.*, 1995) protocols for solid-phase peptide synthesis, although other methods, including a modified Merrifield method, have been used (Christensen *et al.*, 1995).

 PNA monomers or ready-made oligomers are commercially available from PerSeptive Biosystems (Framingham, MA). PNA syntheses by either Boc or Fmoc 15 protocols are straightforward using manual or automated protocols (Norton *et al.*, 1995). The manual protocol lends itself to the production of chemically modified PNAs or the simultaneous synthesis of families of closely related PNAs.

 As with peptide synthesis, the success of a particular PNA synthesis will depend on the properties of the chosen sequence. For example, while in theory PNAs can 20 incorporate any combination of nucleotide bases, the presence of adjacent purines can lead to deletions of one or more residues in the product. In expectation of this difficulty, it is suggested that, in producing PNAs with adjacent purines, one should repeat the coupling of residues likely to be added inefficiently. This should be followed by the purification of PNAs by reverse-phase high-pressure liquid chromatography (Norton *et al.*, 1995) 25 providing yields and purity of product similar to those observed during the synthesis of peptides.

 Modifications of PNAs for a given application may be accomplished by coupling amino acids during solid-phase synthesis or by attaching compounds that contain a carboxylic acid group to the exposed N-terminal amine. Alternatively, PNAs can be

In contrast to DNA and RNA, which contain negatively charged linkages, the PNA backbone is neutral. In spite of this dramatic alteration, PNAs recognize complementary DNA and RNA by Watson-Crick pairing (Egholm *et al.*, 1993), validating the initial modeling by Nielsen *et al.* (1991). PNAs lack 3' to 5' polarity and can bind in either parallel or antiparallel fashion, with the antiparallel mode being preferred (Egholm *et al.*, 1993).

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One might expect that tight binding of PNAs to complementary sequences would also increase binding to similar (but not identical) sequences, reducing the sequence specificity of PNA recognition. As with DNA hybridization, however, selective recognition can be achieved by balancing oligomer length and incubation temperature.

5 Moreover, selective hybridization of PNAs is encouraged by PNA-DNA hybridization being less tolerant of base mismatches than DNA-DNA hybridization. For example, a single mismatch within a 16 bp PNA-DNA duplex can reduce the T_m by up to 15°C (Egholm *et al.*, 1993). This high level of discrimination has allowed the development of several PNA-based strategies for the analysis of point mutations (Wang *et al.*, 1996;

10 Carlsson *et al.*, 1996; Thiede *et al.*, 1996; Webb and Hurskainen, 1996; Perry-O'Keefe *et al.*, 1996).

High-affinity binding provides clear advantages for molecular recognition and the development of new applications for PNAs. For example, 11-13 nucleotide PNAs inhibit the activity of telomerase, a ribonucleo-protein that extends telomere ends using an

15 essential RNA template, while the analogous DNA oligomers do not (Norton *et al.*, 1996).

Neutral PNAs are more hydrophobic than analogous DNA oligomers, and this can lead to difficulty solubilizing them at neutral pH, especially if the PNAs have a high purine content or if they have the potential to form secondary structures. Their solubility can be enhanced by attaching one or more positive charges to the PNA termini

20 (Nielsen *et al.*, 1991).

Findings by Allfrey and colleagues suggest that strand invasion will occur spontaneously at sequences within chromosomal DNA (Boffa *et al.*, 1995; Boffa *et al.*, 1996). These studies targeted PNAs to triplet repeats of the nucleotides CAG and used this recognition to purify transcriptionally active DNA (Boffa *et al.*, 1995) and to inhibit

25 transcription (Boffa *et al.*, 1996). This result suggests that if PNAs can be delivered within cells then they will have the potential to be general sequence-specific regulators of gene expression. Studies and reviews concerning the use of PNAs as antisense and anti-gene agents include Nielsen *et al.* (1993b), Hanvey *et al.* (1992), and Good and Nielsen (1997).

Koppelhus *et al.* (1997) have used PNAs to inhibit HIV-1 inverse transcription, showing that PNAs may be used for antiviral therapies.

Methods of characterizing the antisense binding properties of PNAs are discussed in Rose (1993) and Jensen *et al.* (1997). Rose uses capillary gel electrophoresis to determine binding of PNAs to their complementary oligonucleotide, measuring the relative binding kinetics and stoichiometry. Similar types of measurements were made by Jensen *et al.* using BIAcore™ technology.

Other applications of PNAs include use in DNA strand invasion (Nielsen *et al.*, 1991), antisense inhibition (Hanvey *et al.*, 1992), mutational analysis (Orum *et al.*, 1993), enhancers of transcription (Mollegaard *et al.*, 1994), nucleic acid purification (Orum *et al.*, 1995), isolation of transcriptionally active genes (Boffa *et al.*, 1995), blocking of transcription factor binding (Vickers *et al.*, 1995), genome cleavage (Veselkov *et al.*, 1996), biosensors (Wang *et al.*, 1996), *in situ* hybridization (Thisted *et al.*, 1996), and in a alternative to Southern blotting (Perry-O'Keefe, 1996).

15 POLYPEPTIDE COMPOSITIONS

The present invention, in other aspects, provides polypeptide compositions. Generally, a polypeptide of the invention will be an isolated polypeptide (or an epitope, variant, or active fragment thereof) derived from a mammalian species. Preferably, the polypeptide is encoded by a polynucleotide sequence disclosed herein or a sequence which hybridizes under moderately stringent conditions to a polynucleotide sequence disclosed herein. Alternatively, the polypeptide may be defined as a polypeptide which comprises a contiguous amino acid sequence from an amino acid sequence disclosed herein, or which polypeptide comprises an entire amino acid sequence disclosed herein.

In the present invention, a polypeptide composition is also understood to comprise one or more polypeptides that are immunologically reactive with antibodies generated against a polypeptide of the invention, particularly a polypeptide having the amino acid sequence disclosed in SEQ ID NO: 786, 787, 791, 793, 795, 797-799, 806, 809,

1670-1675, or to active fragments, or to variants or biological functional equivalents thereof.

Likewise, a polypeptide composition of the present invention is understood to comprise one or more polypeptides that are capable of eliciting antibodies that are immunologically reactive with one or more polypeptides encoded by one or more contiguous nucleic acid sequences contained in SEQ ID NO: 1, 11-13, 15, 20, 23-27, 29, 30, 33, 34, 39, 41, 43-46, 51, 52, 57, 58, 60, 62, 65-67, 69-71, 74, 76, 79, 80, 84, 86, 89-92, 95, 97, 98, 101, 110, 111, 113-119, 121-128, 130-134, 136, 138, 139, 141, 143, 146-151, 153, 154, 157-160, 162-164, 167-178, 180, 181, 183, 186-190, 192, 193, 195-220, 224, 226-231, 234, 236, 237, 240, 241, 244-246, 248, 254, 255, 261, 262, 266, 270, 275, 280, 282, 283, 288, 289, 290, 292, 295, 301, 303, 304, 309, 311, 341-782, 784, 785, 790, 792, 794, 796, 800-804, 807, 808, 810-826, 828-1664, 1669 and 1676 or to active fragments, or to variants thereof, or to one or more nucleic acid sequences which hybridize to one or more of these sequences under conditions of moderate to high stringency. Particularly illustrative polypeptides include the amino acid sequences disclosed in SEQ ID NO: 786, 787, 791, 793, 795, 797-799, 806, 809, 827 and 1670-1675..

As used herein, an active fragment of a polypeptide includes a whole or a portion of a polypeptide which is modified by conventional techniques, *e.g.*, mutagenesis, or by addition, deletion, or substitution, but which active fragment exhibits substantially the same structure function, antigenicity, etc., as a polypeptide as described herein.

In certain illustrative embodiments, the polypeptides of the invention will comprise at least an immunogenic portion of a lung tumor protein or a variant thereof, as described herein. As noted above, a "lung tumor protein" is a protein that is expressed by lung tumor cells. Proteins that are lung tumor proteins also react detectably within an immunoassay (such as an ELISA) with antisera from a patient with lung cancer. Polypeptides as described herein may be of any length. Additional sequences derived from the native protein and/or heterologous sequences may be present, and such sequences may (but need not) possess further immunogenic or antigenic properties.

An "immunogenic portion," as used herein is a portion of a protein that is recognized (*i.e.*, specifically bound) by a B-cell and/or T-cell surface antigen receptor. Such immunogenic portions generally comprise at least 5 amino acid residues, more preferably at least 10, and still more preferably at least 20 amino acid residues of a lung tumor protein or a variant thereof. Certain preferred immunogenic portions include peptides in which an N-terminal leader sequence and/or transmembrane domain have been deleted. Other preferred immunogenic portions may contain a small N- and/or C-terminal deletion (*e.g.*, 1-30 amino acids, preferably 5-15 amino acids), relative to the mature protein.

Immunogenic portions may generally be identified using well known techniques, such as those summarized in Paul, *Fundamental Immunology*, 3rd ed., 243-247 (Raven Press, 1993) and references cited therein. Such techniques include screening polypeptides for the ability to react with antigen-specific antibodies, antisera and/or T-cell lines or clones. As used herein, antisera and antibodies are "antigen-specific" if they specifically bind to an antigen (*i.e.*, they react with the protein in an ELISA or other immunoassay, and do not react detectably with unrelated proteins). Such antisera and antibodies may be prepared as described herein, and using well known techniques. An immunogenic portion of a native lung tumor protein is a portion that reacts with such antisera and/or T-cells at a level that is not substantially less than the reactivity of the full length polypeptide (*e.g.*, in an ELISA and/or T-cell reactivity assay). Such immunogenic portions may react within such assays at a level that is similar to or greater than the reactivity of the full length polypeptide. Such screens may generally be performed using methods well known to those of ordinary skill in the art, such as those described in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. For example, a polypeptide may be immobilized on a solid support and contacted with patient sera to allow binding of antibodies within the sera to the immobilized polypeptide. Unbound sera may then be removed and bound antibodies detected using, for example, ¹²⁵I-labeled Protein A.

5 In other words, the ability of a variant to react with antigen-specific antisera may be enhanced or unchanged, relative to the native protein, or may be diminished by less than 50%, and preferably less than 20%, relative to the native protein. Such variants may generally be identified by modifying one of the above polypeptide sequences and evaluating the reactivity of the modified polypeptide with antigen-specific antibodies or
10 antisera as described herein. Preferred variants include those in which one or more portions, such as an N-terminal leader sequence or transmembrane domain, have been removed. Other preferred variants include variants in which a small portion (*e.g.*, 1-30 amino acids, preferably 5-15 amino acids) has been removed from the N- and/or C-terminal of the mature protein.

Preferably, a variant contains conservative substitutions. A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydrophobic nature of the polypeptide to be substantially unchanged. Amino acid substitutions may generally be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative

changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. A variant may also, or alternatively, contain nonconservative changes. In a preferred embodiment, variant polypeptides differ from a native sequence by substitution, deletion or addition of five amino acids or fewer. Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the immunogenicity, secondary structure and hydropathic nature of the polypeptide.

As noted above, polypeptides may comprise a signal (or leader) sequence at the N-terminal end of the protein, which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (e.g., poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

Polypeptides may be prepared using any of a variety of well known techniques. Recombinant polypeptides encoded by DNA sequences as described above may be readily prepared from the DNA sequences using any of a variety of expression vectors known to those of ordinary skill in the art. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast, and higher eukaryotic cells, such as mammalian cells and plant cells. Preferably, the host cells employed are *E. coli*, yeast or a mammalian cell line such as COS or CHO. Supernatants from suitable host/vector systems which secrete recombinant protein or polypeptide into culture media may be first concentrated using a commercially available filter. Following concentration, the concentrate may be applied to a suitable purification matrix such as an affinity matrix or an ion exchange resin. Finally, one or more reverse phase HPLC steps can be employed to further purify a recombinant polypeptide.

Portions and other variants having less than about 100 amino acids, and generally less than about 50 amino acids, may also be generated by synthetic means, using

techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. See Merrifield, *J. Am. Chem. Soc.* 85:2149-2146, 1963.

- 5 Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied BioSystems Division (Foster City, CA), and may be operated according to the manufacturer's instructions.

Within certain specific embodiments, a polypeptide may be a fusion protein that comprises multiple polypeptides as described herein, or that comprises at least one
10 polypeptide as described herein and an unrelated sequence, such as a known tumor protein. A fusion partner may, for example, assist in providing T helper epitopes (an immunological fusion partner), preferably T helper epitopes recognized by humans, or may assist in expressing the protein (an expression enhancer) at higher yields than the native recombinant protein. Certain preferred fusion partners are both immunological and
15 expression enhancing fusion partners. Other fusion partners may be selected so as to increase the solubility of the protein or to enable the protein to be targeted to desired intracellular compartments. Still further fusion partners include affinity tags, which facilitate purification of the protein.

Fusion proteins may generally be prepared using standard techniques,
20 including chemical conjugation. Preferably, a fusion protein is expressed as a recombinant protein, allowing the production of increased levels, relative to a non-fused protein, in an expression system. Briefly, DNA sequences encoding the polypeptide components may be assembled separately, and ligated into an appropriate expression vector. The 3' end of the DNA sequence encoding one polypeptide component is ligated, with or without a peptide
25 linker, to the 5' end of a DNA sequence encoding the second polypeptide component so that the reading frames of the sequences are in phase. This permits translation into a single fusion protein that retains the biological activity of both component polypeptides.

A peptide linker sequence may be employed to separate the first and second polypeptide components by a distance sufficient to ensure that each polypeptide folds into

its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the fusion protein using standard techniques well known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea *et al.*, *Gene* 40:39-46, 1985; Murphy *et al.*, *Proc. Natl. Acad. Sci. USA* 83:8258-8262, 1986; U.S. Patent No. 4,935,233 and U.S. Patent No. 4,751,180. The linker sequence may generally be from 1 to about 50 amino acids in length. Linker sequences are not required when the first and second polypeptides have non-essential N-terminal amino acid regions that can be used to separate the functional domains and prevent steric interference.

The ligated DNA sequences are operably linked to suitable transcriptional or translational regulatory elements. The regulatory elements responsible for expression of DNA are located only 5' to the DNA sequence encoding the first polypeptides. Similarly, stop codons required to end translation and transcription termination signals are only present 3' to the DNA sequence encoding the second polypeptide.

Fusion proteins are also provided. Such proteins comprise a polypeptide as described herein together with an unrelated immunogenic protein. Preferably the immunogenic protein is capable of eliciting a recall response. Examples of such proteins include tetanus, tuberculosis and hepatitis proteins (*see*, for example, Stoute *et al.* *New Engl. J. Med.*, 336:86-91, 1997).

Within preferred embodiments, an immunological fusion partner is derived from protein D, a surface protein of the gram-negative bacterium *Haemophilus influenza B* (WO 91/18926). Preferably, a protein D derivative comprises approximately the first third of the protein (*e.g.*, the first N-terminal 100-110 amino acids), and a protein D derivative may be lipidated. Within certain preferred embodiments, the first 109 residues of a

Lipoprotein D fusion partner is included on the N-terminus to provide the polypeptide with additional exogenous T-cell epitopes and to increase the expression level in *E. coli* (thus functioning as an expression enhancer). The lipid tail ensures optimal presentation of the antigen to antigen presenting cells. Other fusion partners include the non-structural protein from influenzae virus, NS1 (hemagglutinin). Typically, the N-terminal 81 amino acids are used, although different fragments that include T-helper epitopes may be used.

In another embodiment, the immunological fusion partner is the protein known as LYTA, or a portion thereof (preferably a C-terminal portion). LYTA is derived from *Streptococcus pneumoniae*, which synthesizes an N-acetyl-L-alanine amidase known as amidase LYTA (encoded by the *LytA* gene; *Gene* 43:265-292, 1986). LYTA is an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LYTA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of *E. coli* C-LYTA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C-LYTA fragment at the amino terminus has been described (see *Biotechnology* 10:795-798, 1992). Within a preferred embodiment, a repeat portion of LYTA may be incorporated into a fusion protein. A repeat portion is found in the C-terminal region starting at residue 178. A particularly preferred repeat portion incorporates residues 188-305.

In general, polypeptides (including fusion proteins) and polynucleotides as described herein are isolated. An "isolated" polypeptide or polynucleotide is one that is removed from its original environment. For example, a naturally-occurring protein is isolated if it is separated from some or all of the coexisting materials in the natural system. Preferably, such polypeptides are at least about 90% pure, more preferably at least about 95% pure and most preferably at least about 99% pure. A polynucleotide is considered to be isolated if, for example, it is cloned into a vector that is not a part of the natural environment.

BINDING AGENTS

The present invention further provides agents, such as antibodies and antigen-binding fragments thereof, that specifically bind to a lung tumor protein. As used herein, an antibody, or antigen-binding fragment thereof, is said to "specifically bind" to a lung tumor protein if it reacts at a detectable level (within, for example, an ELISA) with a lung tumor protein, and does not react detectably with unrelated proteins under similar conditions. As used herein, "binding" refers to a noncovalent association between two separate molecules such that a complex is formed. The ability to bind may be evaluated by, for example, determining a binding constant for the formation of the complex. The binding constant is the value obtained when the concentration of the complex is divided by the product of the component concentrations. In general, two compounds are said to "bind," in the context of the present invention, when the binding constant for complex formation exceeds about 10^3 L/mol. The binding constant may be determined using methods well known in the art.

Binding agents may be further capable of differentiating between patients with and without a cancer, such as lung cancer, using the representative assays provided herein. In other words, antibodies or other binding agents that bind to a lung tumor protein will generate a signal indicating the presence of a cancer in at least about 20% of patients with the disease, and will generate a negative signal indicating the absence of the disease in at least about 90% of individuals without the cancer. To determine whether a binding agent satisfies this requirement, biological samples (*e.g.*, blood, sera, sputum, urine and/or tumor biopsies) from patients with and without a cancer (as determined using standard clinical tests) may be assayed as described herein for the presence of polypeptides that bind to the binding agent. It will be apparent that a statistically significant number of samples with and without the disease should be assayed. Each binding agent should satisfy the above criteria; however, those of ordinary skill in the art will recognize that binding agents may be used in combination to improve sensitivity.

Any agent that satisfies the above requirements may be a binding agent. For example, a binding agent may be a ribosome, with or without a peptide component, an

RNA molecule or a polypeptide. In a preferred embodiment, a binding agent is an antibody or an antigen-binding fragment thereof. Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art. See, e.g., Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In
5 general, antibodies can be produced by cell culture techniques, including the generation of monoclonal antibodies as described herein, or via transfection of antibody genes into suitable bacterial or mammalian cell hosts, in order to allow for the production of recombinant antibodies. In one technique, an immunogen comprising the polypeptide is initially injected into any of a wide variety of mammals (e.g., mice, rats, rabbits, sheep or
10 goats). In this step, the polypeptides of this invention may serve as the immunogen without modification. Alternatively, particularly for relatively short polypeptides, a superior immune response may be elicited if the polypeptide is joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating one or more
15 booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for an antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol.*
20 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (i.e., reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell
25 fusion partner, preferably one that is syngeneic with the immunized animal. A variety of fusion techniques may be employed. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection.

Monoclonal antibodies may be isolated from the supernatants of growing

Within certain embodiments, the use of antigen-binding fragments of antibodies may be preferred. Such fragments include Fab fragments, which may be prepared using standard techniques. Briefly, immunoglobulins may be purified from rabbit serum by affinity chromatography on Protein A bead columns (Harlow and Lane, 15 *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988) and digested by papain to yield Fab and Fc fragments. The Fab and Fc fragments may be separated by affinity chromatography on protein A bead columns.

A therapeutic agent may be coupled (*e.g.*, covalently bonded) to a suitable monoclonal antibody either directly or indirectly (*e.g.*, via a linker group). A direct reaction between an agent and an antibody is possible when each possesses a substituent

capable of reacting with the other. For example, a nucleophilic group, such as an amino or sulfhydryl group, on one may be capable of reacting with a carbonyl-containing group, such as an anhydride or an acid halide, or with an alkyl group containing a good leaving group (*e.g.*, a halide) on the other.

- 5 Alternatively, it may be desirable to couple a therapeutic agent and an antibody via a linker group. A linker group can function as a spacer to distance an antibody from an agent in order to avoid interference with binding capabilities. A linker group can also serve to increase the chemical reactivity of a substituent on an agent or an antibody, and thus increase the coupling efficiency. An increase in chemical reactivity
10 may also facilitate the use of agents, or functional groups on agents, which otherwise would not be possible.

- It will be evident to those skilled in the art that a variety of bifunctional or polyfunctional reagents, both homo- and hetero-functional (such as those described in the catalog of the Pierce Chemical Co., Rockford, IL), may be employed as the linker group.
15 Coupling may be effected, for example, through amino groups, carboxyl groups, sulfhydryl groups or oxidized carbohydrate residues. There are numerous references describing such methodology, *e.g.*, U.S. Patent No. 4,671,958, to Rodwell *et al.*

- Where a therapeutic agent is more potent when free from the antibody portion of the immunoconjugates of the present invention, it may be desirable to use a
20 linker group which is cleavable during or upon internalization into a cell. A number of different cleavable linker groups have been described. The mechanisms for the intracellular release of an agent from these linker groups include cleavage by reduction of a disulfide bond (*e.g.*, U.S. Patent No. 4,489,710, to Spitler), by irradiation of a photolabile bond (*e.g.*, U.S. Patent No. 4,625,014, to Senter *et al.*), by hydrolysis of derivatized amino
25 acid side chains (*e.g.*, U.S. Patent No. 4,638,045, to Kohn *et al.*), by serum complement-mediated hydrolysis (*e.g.*, U.S. Patent No. 4,671,958, to Rodwell *et al.*), and acid-catalyzed hydrolysis (*e.g.*, U.S. Patent No. 4,569,789, to Blattler *et al.*).

 It may be desirable to couple more than one agent to an antibody. In one embodiment, multiple molecules of an agent are coupled to one antibody molecule. In

another embodiment, more than one type of agent may be coupled to one antibody. Regardless of the particular embodiment, immunoconjugates with more than one agent may be prepared in a variety of ways. For example, more than one agent may be coupled directly to an antibody molecule, or linkers that provide multiple sites for attachment can
5 be used. Alternatively, a carrier can be used.

A carrier may bear the agents in a variety of ways, including covalent bonding either directly or via a linker group. Suitable carriers include proteins such as albumins (*e.g.*, U.S. Patent No. 4,507,234, to Kato *et al.*), peptides and polysaccharides such as aminodextran (*e.g.*, U.S. Patent No. 4,699,784, to Shih *et al.*). A carrier may also
10 bear an agent by noncovalent bonding or by encapsulation, such as within a liposome vesicle (*e.g.*, U.S. Patent Nos. 4,429,008 and 4,873,088). Carriers specific for radionuclide agents include radiohalogenated small molecules and chelating compounds. For example, U.S. Patent No. 4,735,792 discloses representative radiohalogenated small molecules and their synthesis. A radionuclide chelate may be formed from chelating compounds that
15 include those containing nitrogen and sulfur atoms as the donor atoms for binding the metal, or metal oxide, radionuclide. For example, U.S. Patent No. 4,673,562, to Davison *et al.* discloses representative chelating compounds and their synthesis.

A variety of routes of administration for the antibodies and immunoconjugates may be used. Typically, administration will be intravenous,
20 intramuscular, subcutaneous or in the bed of a resected tumor. It will be evident that the precise dose of the antibody/immunoconjugate will vary depending upon the antibody used, the antigen density on the tumor, and the rate of clearance of the antibody.

T CELLS

Immunotherapeutic compositions may also, or alternatively, comprise T
25 cells specific for a lung tumor protein. Such cells may generally be prepared *in vitro* or *ex vivo*, using standard procedures. For example, T cells may be isolated from bone marrow, peripheral blood, or a fraction of bone marrow or peripheral blood of a patient, using a commercially available cell separation system, such as the Isolex™ System, available from

Nexell Therapeutics, Inc. (Irvine, CA; see also U.S. Patent No. 5,240,856; U.S. Patent No. 5,215,926; WO 89/06280; WO 91/16116 and WO 92/07243). Alternatively, T cells may be derived from related or unrelated humans, non-human mammals, cell lines or cultures.

- 5 T cells may be stimulated with a lung tumor polypeptide, polynucleotide encoding a lung tumor polypeptide and/or an antigen presenting cell (APC) that expresses such a polypeptide. Such stimulation is performed under conditions and for a time sufficient to permit the generation of T cells that are specific for the polypeptide. Preferably, a lung tumor polypeptide or polynucleotide is present within a delivery vehicle, such as a microsphere, to facilitate the generation of specific T cells.
- 10 T cells are considered to be specific for a lung tumor polypeptide if the T cells specifically proliferate, secrete cytokines or kill target cells coated with the polypeptide or expressing a gene encoding the polypeptide. T cell specificity may be evaluated using any of a variety of standard techniques. For example, within a chromium release assay or proliferation assay, a stimulation index of more than two fold increase in
- 15 lysis and/or proliferation, compared to negative controls, indicates T cell specificity. Such assays may be performed, for example, as described in Chen *et al.*, *Cancer Res.* 54:1065-1070, 1994. Alternatively, detection of the proliferation of T cells may be accomplished by a variety of known techniques. For example, T cell proliferation can be detected by measuring an increased rate of DNA synthesis (*e.g.*, by pulse-labeling cultures of T cells
- 20 with tritiated thymidine and measuring the amount of tritiated thymidine incorporated into DNA). Contact with a lung tumor polypeptide (100 ng/ml - 100 µg/ml, preferably 200 ng/ml - 25 µg/ml) for 3 - 7 days should result in at least a two fold increase in proliferation of the T cells. Contact as described above for 2-3 hours should result in activation of the T cells, as measured using standard cytokine assays in which a two fold increase in the level
- 25 of cytokine release (*e.g.*, TNF or IFN-γ) is indicative of T cell activation (*see* Coligan *et al.*, *Current Protocols in Immunology*, vol. 1, Wiley Interscience (Greene 1998)). T cells that have been activated in response to a lung tumor polypeptide, polynucleotide or polypeptide-expressing APC may be CD4⁺ and/or CD8⁺. Lung tumor protein-specific T cells may be expanded using standard techniques. Within preferred embodiments, the T

cells are derived from a patient, a related donor or an unrelated donor, and are administered to the patient following stimulation and expansion.

For therapeutic purposes, CD4⁺ or CD8⁺ T cells that proliferate in response to a lung tumor polypeptide, polynucleotide or APC can be expanded in number either
5 *in vitro* or *in vivo*. Proliferation of such T cells *in vitro* may be accomplished in a variety of ways. For example, the T cells can be re-exposed to a lung tumor polypeptide, or a short peptide corresponding to an immunogenic portion of such a polypeptide, with or without the addition of T cell growth factors, such as interleukin-2, and/or stimulator cells that synthesize a lung tumor polypeptide. Alternatively, one or more T cells that proliferate in
10 the presence of a lung tumor protein can be expanded in number by cloning. Methods for cloning cells are well known in the art, and include limiting dilution.

PHARMACEUTICAL COMPOSITIONS

In additional embodiments, the present invention concerns formulation of one or more of the polynucleotide, polypeptide, T-cell and/or antibody compositions
15 disclosed herein in pharmaceutically-acceptable solutions for administration to a cell or an animal, either alone, or in combination with one or more other modalities of therapy.

It will also be understood that, if desired, the nucleic acid segment, RNA, DNA or PNA compositions that express a polypeptide as disclosed herein may be administered in combination with other agents as well, such as, *e.g.*, other proteins or
20 polypeptides or various pharmaceutically-active agents. In fact, there is virtually no limit to other components that may also be included, given that the additional agents do not cause a significant adverse effect upon contact with the target cells or host tissues. The compositions may thus be delivered along with various other agents as required in the particular instance. Such compositions may be purified from host cells or other biological
25 sources, or alternatively may be chemically synthesized as described herein. Likewise, such compositions may further comprise substituted or derivatized RNA or DNA compositions.

Formulation of pharmaceutically-acceptable excipients and carrier solutions is well-known to those of skill in the art, as is the development of suitable dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens, including *e.g.*, oral, parenteral, intravenous, intranasal, and intramuscular administration and formulation.

1. ORAL DELIVERY

In certain applications, the pharmaceutical compositions disclosed herein may be delivered *via* oral administration to an animal. As such, these compositions may be formulated with an inert diluent or with an assimilable edible carrier, or they may be enclosed in hard- or soft-shell gelatin capsule, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet.

The active compounds may even be incorporated with excipients and used in the form of ingestible tablets, buccal tables, troches, capsules, elixirs, suspensions, syrups, wafers, and the like (Mathiowitz *et al.*, 1997; Hwang *et al.*, 1998; U. S. Patent 5,641,515; U. S. Patent 5,580,579 and U. S. Patent 5,792,451, each specifically incorporated herein by reference in its entirety). The tablets, troches, pills, capsules and the like may also contain the following: a binder, as gum tragacanth, acacia, cornstarch, or gelatin; excipients, such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid and the like; a lubricant, such as magnesium stearate; and a sweetening agent, such as sucrose, lactose or saccharin may be added or a flavoring agent, such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar, or both. A syrup of elixir may contain the active compound sucrose as a sweetening agent methyl and propylparabens as preservatives, a dye and flavoring, such as cherry or orange flavor. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition,

the active compounds may be incorporated into sustained-release preparation and formulations.

Typically, these formulations may contain at least about 0.1% of the active compound or more, although the percentage of the active ingredient(s) may, of course, be varied and may conveniently be between about 1 or 2% and about 60% or 70% or more of the weight or volume of the total formulation. Naturally, the amount of active compound(s) in each therapeutically useful composition may be prepared in such a way that a suitable dosage will be obtained in any given unit dose of the compound. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in the art of preparing such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable.

For oral administration the compositions of the present invention may alternatively be incorporated with one or more excipients in the form of a mouthwash, dentifrice, buccal tablet, oral spray, or sublingual orally-administered formulation. For example, a mouthwash may be prepared incorporating the active ingredient in the required amount in an appropriate solvent, such as a sodium borate solution (Dobell's Solution). Alternatively, the active ingredient may be incorporated into an oral solution such as one containing sodium borate, glycerin and potassium bicarbonate, or dispersed in a dentifrice, or added in a therapeutically-effective amount to a composition that may include water, binders, abrasives, flavoring agents, foaming agents, and humectants. Alternatively the compositions may be fashioned into a tablet or solution form that may be placed under the tongue or otherwise dissolved in the mouth.

2. INJECTABLE DELIVERY

In certain circumstances it will be desirable to deliver the pharmaceutical compositions disclosed herein parenterally, intravenously, intramuscularly, or even intraperitoneally as described in U. S. Patent 5,543,158; U. S. Patent 5,641,515 and U. S. Patent 5,399,363 (each specifically incorporated herein by reference in its entirety).

Solutions of the active compounds as free base or pharmacologically acceptable salts may be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations
5 contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions (U. S. Patent 5,466,468, specifically incorporated herein by reference in its entirety). In all cases the form must be sterile and must be fluid to the
10 extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper
15 fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be facilitated by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic
20 agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered
25 isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, a sterile aqueous medium that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or

injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, and the general safety and purity standards as required by FDA Office of Biologics standards.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The compositions disclosed herein may be formulated in a neutral or salt form. Pharmaceutically-acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions, drug-release capsules, and the like.

As used herein, "carrier" includes any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption

delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The phrase "pharmaceutically-acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human. The preparation of an aqueous composition that contains a protein as an active ingredient is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified.

3. NASAL DELIVERY

In certain embodiments, the pharmaceutical compositions may be delivered by intranasal sprays, inhalation, and/or other aerosol delivery vehicles. Methods for delivering genes, nucleic acids, and peptide compositions directly to the lungs *via* nasal aerosol sprays has been described *e.g.*, in U. S. Patent 5,756,353 and U. S. Patent 5,804,212 (each specifically incorporated herein by reference in its entirety). Likewise, the delivery of drugs using intranasal microparticle resins (Takenaga *et al.*, 1998) and lysophosphatidyl-glycerol compounds (U. S. Patent 5,725,871, specifically incorporated herein by reference in its entirety) are also well-known in the pharmaceutical arts. Likewise, transmucosal drug delivery in the form of a polytetrafluoroethylene support matrix is described in U. S. Patent 5,780,045 (specifically incorporated herein by reference in its entirety).

4. LIPOSOME-, NANOCAPSULE-, AND MICROPARTICLE-MEDIATED DELIVERY

In certain embodiments, the inventors contemplate the use of liposomes, nanocapsules, microparticles, microspheres, lipid particles, vesicles, and the like, for the introduction of the compositions of the present invention into suitable host cells. In

particular, the compositions of the present invention may be formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, a nanosphere, or a nanoparticle or the like.

Such formulations may be preferred for the introduction of
 5 pharmaceutically-acceptable formulations of the nucleic acids or constructs disclosed herein. The formation and use of liposomes is generally known to those of skill in the art (see for example, Couvreur *et al.*, 1977; Couvreur, 1988; Lasic, 1998; which describes the use of liposomes and nanocapsules in the targeted antibiotic therapy for intracellular bacterial infections and diseases). Recently, liposomes were developed with improved
 10 serum stability and circulation half-times (Gabizon and Papahadjopoulos, 1988; Allen and Choun, 1987; U. S. Patent 5,741,516, specifically incorporated herein by reference in its entirety). Further, various methods of liposome and liposome like preparations as potential drug carriers have been reviewed (Takakura, 1998; Chandran *et al.*, 1997; Margalit, 1995; U. S. Patent 5,567,434; U. S. Patent 5,552,157; U. S. Patent 5,565,213; U. S. Patent
 15 5,738,868 and U. S. Patent 5,795,587, each specifically incorporated herein by reference in its entirety).

Liposomes have been used successfully with a number of cell types that are normally resistant to transfection by other procedures including T cell suspensions, primary hepatocyte cultures and PC 12 cells (Renneisen *et al.*, 1990; Muller *et al.*, 1990). In
 20 addition, liposomes are free of the DNA length constraints that are typical of viral-based delivery systems. Liposomes have been used effectively to introduce genes, drugs (Heath and Martin, 1986; Heath *et al.*, 1986; Balazsovits *et al.*, 1989; Fresta and Puglisi, 1996), radiotherapeutic agents (Pikul *et al.*, 1987), enzymes (Imaizumi *et al.*, 1990a; Imaizumi
 25 *et al.*, 1990b), viruses (Faller and Baltimore, 1984), transcription factors and allosteric effectors (Nicolau and Gersonde, 1979) into a variety of cultured cell lines and animals. In addition, several successful clinical trails examining the effectiveness of liposome-mediated drug delivery have been completed (Lopez-Berestein *et al.*, 1985a; 1985b; Coune, 1988; Sculier *et al.*, 1988). Furthermore, several studies suggest that the use of

liposomes is not associated with autoimmune responses, toxicity or gonadal localization after systemic delivery (Mori and Fukatsu, 1992).

Liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed
5 multilamellar vesicles (MLVs). MLVs generally have diameters of from 25 nm to 4 μ m. Sonication of MLVs results in the formation of small unilamellar vesicles (SUVs) with diameters in the range of 200 to 500 Å, containing an aqueous solution in the core.

Liposomes bear resemblance to cellular membranes and are contemplated for use in connection with the present invention as carriers for the peptide compositions.
10 They are widely suitable as both water- and lipid-soluble substances can be entrapped, *i.e.* in the aqueous spaces and within the bilayer itself, respectively. It is possible that the drug-bearing liposomes may even be employed for site-specific delivery of active agents by selectively modifying the liposomal formulation.

In addition to the teachings of Couvreur *et al.* (1977; 1988), the following
15 information may be utilized in generating liposomal formulations. Phospholipids can form a variety of structures other than liposomes when dispersed in water, depending on the molar ratio of lipid to water. At low ratios the liposome is the preferred structure. The physical characteristics of liposomes depend on pH, ionic strength and the presence of divalent cations. Liposomes can show low permeability to ionic and polar substances, but
20 at elevated temperatures undergo a phase transition which markedly alters their permeability. The phase transition involves a change from a closely packed, ordered structure, known as the gel state, to a loosely packed, less-ordered structure, known as the fluid state. This occurs at a characteristic phase-transition temperature and results in an increase in permeability to ions, sugars and drugs.

25 In addition to temperature, exposure to proteins can alter the permeability of liposomes. Certain soluble proteins, such as cytochrome c, bind, deform and penetrate the bilayer, thereby causing changes in permeability. Cholesterol inhibits this penetration of proteins, apparently by packing the phospholipids more tightly. It is contemplated that the

most useful liposome formations for antibiotic and inhibitor delivery will contain cholesterol.

The ability to trap solutes varies between different types of liposomes. For example, MLVs are moderately efficient at trapping solutes, but SUVs are extremely inefficient. SUVs offer the advantage of homogeneity and reproducibility in size distribution, however, and a compromise between size and trapping efficiency is offered by large unilamellar vesicles (LUVs). These are prepared by ether evaporation and are three to four times more efficient at solute entrapment than MLVs.

In addition to liposome characteristics, an important determinant in entrapping compounds is the physicochemical properties of the compound itself. Polar compounds are trapped in the aqueous spaces and nonpolar compounds bind to the lipid bilayer of the vesicle. Polar compounds are released through permeation or when the bilayer is broken, but nonpolar compounds remain affiliated with the bilayer unless it is disrupted by temperature or exposure to lipoproteins. Both types show maximum efflux rates at the phase transition temperature.

Liposomes interact with cells *via* four different mechanisms: endocytosis by phagocytic cells of the reticuloendothelial system such as macrophages and neutrophils; adsorption to the cell surface, either by nonspecific weak hydrophobic or electrostatic forces, or by specific interactions with cell-surface components; fusion with the plasma membrane by insertion of the lipid bilayer of the liposome into the plasma membrane, with simultaneous release of liposomal contents into the cytoplasm; and by transfer of liposomal lipids to cellular or subcellular membranes, or vice versa, without any association of the liposome contents. It often is difficult to determine which mechanism is operative and more than one may operate at the same time.

The fate and disposition of intravenously injected liposomes depend on their physical properties, such as size, fluidity, and surface charge. They may persist in tissues for h or days, depending on their composition, and half lives in the blood range from min to several h. Larger liposomes, such as MLVs and LUVs, are taken up rapidly by phagocytic cells of the reticuloendothelial system, but physiology of the circulatory system restrains

the exit of such large species at most sites. They can exit only in places where large openings or pores exist in the capillary endothelium, such as the sinusoids of the liver or spleen. Thus, these organs are the predominate site of uptake. On the other hand, SUVs show a broader tissue distribution but still are sequestered highly in the liver and spleen. In
 5 general, this *in vivo* behavior limits the potential targeting of liposomes to only those organs and tissues accessible to their large size. These include the blood, liver, spleen, bone marrow, and lymphoid organs.

Targeting is generally not a limitation in terms of the present invention. However, should specific targeting be desired, methods are available for this to be
 10 accomplished. Antibodies may be used to bind to the liposome surface and to direct the antibody and its drug contents to specific antigenic receptors located on a particular cell-type surface. Carbohydrate determinants (glycoprotein or glycolipid cell-surface components that play a role in cell-cell recognition, interaction and adhesion) may also be used as recognition sites as they have potential in directing liposomes to particular cell
 15 types. Mostly, it is contemplated that intravenous injection of liposomal preparations would be used, but other routes of administration are also conceivable.

Alternatively, the invention provides for pharmaceutically-acceptable nanocapsule formulations of the compositions of the present invention. Nanocapsules can generally entrap compounds in a stable and reproducible way (Henry-Michelland *et al.*,
 20 1987; Quintanar-Guerrero *et al.*, 1998; Douglas *et al.*, 1987). To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 μm) should be designed using polymers able to be degraded *in vivo*. Biodegradable polyalkylcyanoacrylate nanoparticles that meet these requirements are contemplated for use in the present invention. Such particles may be easily made, as described (Couvreur *et al.*,
 25 1980; 1988; zur Muhlen *et al.*, 1998; Zambaux *et al.* 1998; Pinto-Alphandry *et al.*, 1995 and U. S. Patent 5,145,684, specifically incorporated herein by reference in its entirety).

IMMUNOGENIC COMPOSITIONS

In certain preferred embodiments of the present invention, immunogenic compositions, or vaccines, are provided. The immunogenic compositions will generally comprise one or more pharmaceutical compositions, such as those discussed above, in combination with an immunostimulant. An immunostimulant may be any substance that enhances or potentiates an immune response (antibody and/or cell-mediated) to an exogenous antigen. Examples of immunostimulants include adjuvants, biodegradable microspheres (*e.g.*, polylactic galactide) and liposomes (into which the compound is incorporated; *see e.g.*, Fullerton, U.S. Patent No. 4,235,877). Vaccine preparation is generally described in, for example, M.F. Powell and M.J. Newman, eds., "Vaccine Design (the subunit and adjuvant approach)," Plenum Press (NY, 1995). Pharmaceutical compositions and immunogenic compositions, or vaccines, within the scope of the present invention may also contain other compounds, which may be biologically active or inactive. For example, one or more immunogenic portions of other tumor antigens may be present, either incorporated into a fusion polypeptide or as a separate compound, within the composition.

Illustrative immunogenic compositions may contain DNA encoding one or more of the polypeptides as described above, such that the polypeptide is generated *in situ*. As noted above, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacteria and viral expression systems. Numerous gene delivery techniques are well known in the art, such as those described by Rolland, *Crit. Rev. Therap. Drug Carrier Systems* 15:143-198, 1998, and references cited therein. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as *Bacillus-Calmette-Guerrin*) that expresses an immunogenic portion of the polypeptide on its cell surface or secretes such an epitope. In a preferred embodiment, the DNA may be introduced using a viral expression system (*e.g.*, vaccinia or other pox virus, retrovirus, or adenovirus), which may involve the use of a non-

pathogenic (defective), replication competent virus. Suitable systems are disclosed, for example, in Fisher-Hoch *et al.*, *Proc. Natl. Acad. Sci. USA* 86:317-321, 1989; Flexner *et al.*, *Ann. N.Y. Acad. Sci.* 569:86-103, 1989; Flexner *et al.*, *Vaccine* 8:17-21, 1990; U.S. Patent Nos. 4,603,112, 4,769,330, and 5,017,487; WO 89/01973; U.S. Patent

5 No. 4,777,127; GB 2,200,651; EP 0,345,242; WO 91/02805; Berkner, *Biotechniques* 6:616-627, 1988; Rosenfeld *et al.*, *Science* 252:431-434, 1991; Kolls *et al.*, *Proc. Natl. Acad. Sci. USA* 91:215-219, 1994; Kass-Eisler *et al.*, *Proc. Natl. Acad. Sci. USA* 90:11498-11502, 1993; Guzman *et al.*, *Circulation* 88:2838-2848, 1993; and Guzman *et al.*, *Cir. Res.* 73:1202-1207, 1993. Techniques for incorporating DNA into such expression

10 systems are well known to those of ordinary skill in the art. The DNA may also be "naked," as described, for example, in Ulmer *et al.*, *Science* 259:1745-1749, 1993 and reviewed by Cohen, *Science* 259:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells. It will be apparent that an immunogenic composition may comprise both a

15 polynucleotide and a polypeptide component. Such immunogenic compositions may provide for an enhanced immune response.

It will be apparent that an immunogenic composition may contain pharmaceutically acceptable salts of the polynucleotides and polypeptides provided herein. Such salts may be prepared from pharmaceutically acceptable non-toxic bases, including

20 organic bases (*e.g.*, salts of primary, secondary and tertiary amines and basic amino acids) and inorganic bases (*e.g.*, sodium, potassium, lithium, ammonium, calcium and magnesium salts).

While any suitable carrier known to those of ordinary skill in the art may be employed in the immunogenic compositions of this invention, the type of carrier will vary

25 depending on the mode of administration. Compositions of the present invention may be formulated for any appropriate manner of administration, including for example, topical, oral, nasal, intravenous, intracranial, intraperitoneal, subcutaneous or intramuscular administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral

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monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF or interleukin-2, -7, or -12, may also be used as adjuvants.

Within the immunogenic compositions provided herein, the adjuvant composition is preferably designed to induce an immune response predominantly of the Th1 type. High levels of Th1-type cytokines (*e.g.*, IFN- γ , TNF α , IL-2 and IL-12) tend to favor the induction of cell mediated immune responses to an administered antigen. In contrast, high levels of Th2-type cytokines (*e.g.*, IL-4, IL-5, IL-6 and IL-10) tend to favor the induction of humoral immune responses. Following application of an immunogenic composition as provided herein, a patient will support an immune response that includes Th1- and Th2-type responses. Within a preferred embodiment, in which a response is predominantly Th1-type, the level of Th1-type cytokines will increase to a greater extent than the level of Th2-type cytokines. The levels of these cytokines may be readily assessed using standard assays. For a review of the families of cytokines, see Mosmann and Coffman, *Ann. Rev. Immunol.* 7:145-173, 1989.

Preferred adjuvants for use in eliciting a predominantly Th1-type response include, for example, a combination of monophosphoryl lipid A, preferably 3-de-O-acylated monophosphoryl lipid A (3D-MPL), together with an aluminum salt. MPL adjuvants are available from Corixa Corporation (Seattle, WA; *see* US Patent Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. Such oligonucleotides are well known and are described, for example, in WO 96/02555, WO 99/33488 and U.S. Patent Nos. 6,008,200 and 5,856,462. Immunostimulatory DNA sequences are also described, for example, by Sato *et al.*, *Science* 273:352, 1996. Another preferred adjuvant is a saponin, preferably QS21 (Aquila Biopharmaceuticals Inc., Framingham, MA), which may be used alone or in combination with other adjuvants. For example, an enhanced system involves the combination of a monophosphoryl lipid A and saponin derivative, such as the combination of QS21 and 3D-MPL as described in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol, as described in WO 96/33739. Other preferred formulations comprise an oil-in-water

emulsion and tocopherol. A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil-in-water emulsion is described in WO 95/17210.

Other preferred adjuvants include Montanide ISA 720 (Seppic, France), SAF (Chiron, California, United States), ISCOMS (CSL), MF-59 (Chiron), the SBAS series of adjuvants (*e.g.*, SBAS-2 or SBAS-4, available from SmithKline Beecham, Rixensart, Belgium), Detox (Corixa, Hamilton, MT), RC-529 (Corixa, Hamilton, MT) and other aminoalkyl glucosaminide 4-phosphates (AGPs), such as those described in pending U.S. Patent Application Serial Nos. 08/853,826 and 09/074,720, the disclosures of which are incorporated herein by reference in their entireties.

Any immunogenic composition provided herein may be prepared using well known methods that result in a combination of antigen, immune response enhancer and a suitable carrier or excipient. The compositions described herein may be administered as part of a sustained release formulation (*i.e.*, a formulation such as a capsule, sponge or gel (composed of polysaccharides, for example) that effects a slow release of compound following administration). Such formulations may generally be prepared using well known technology (*see, e.g.*, Coombes *et al.*, *Vaccine* 14:1429-1438, 1996) and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain a polypeptide, polynucleotide or antibody dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane.

Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of active component release. Such carriers include microparticles of poly(lactide-co-glycolide), polyacrylate, latex, starch, cellulose, dextran and the like. Other delayed-release carriers include supramolecular biovectors, which comprise a non-liquid hydrophilic core (*e.g.*, a cross-linked polysaccharide or oligosaccharide) and, optionally, an external layer comprising an amphiphilic compound, such as a phospholipid (*see e.g.*, U.S. Patent No. 5,151,254 and PCT applications WO 94/20078, WO/94/23701 and WO 96/06638). The amount of active compound contained within a sustained release formulation depends upon

the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

Any of a variety of delivery vehicles may be employed within pharmaceutical compositions and immunogenic compositions to facilitate production of an antigen-specific immune response that targets tumor cells. Delivery vehicles include antigen presenting cells (APCs), such as dendritic cells, macrophages, B cells, monocytes and other cells that may be engineered to be efficient APCs. Such cells may, but need not, be genetically modified to increase the capacity for presenting the antigen, to improve activation and/or maintenance of the T cell response, to have anti-tumor effects *per se* and/or to be immunologically compatible with the receiver (*i.e.*, matched HLA haplotype). APCs may generally be isolated from any of a variety of biological fluids and organs, including tumor and peritumoral tissues, and may be autologous, allogeneic, syngeneic or xenogeneic cells.

Certain preferred embodiments of the present invention use dendritic cells or progenitors thereof as antigen-presenting cells. Dendritic cells are highly potent APCs (Banchereau and Steinman, *Nature* 392:245-251, 1998) and have been shown to be effective as a physiological adjuvant for eliciting prophylactic or therapeutic antitumor immunity (*see* Timmerman and Levy, *Ann. Rev. Med.* 50:507-529, 1999). In general, dendritic cells may be identified based on their typical shape (stellate *in situ*, with marked cytoplasmic processes (dendrites) visible *in vitro*), their ability to take up, process and present antigens with high efficiency and their ability to activate naïve T cell responses. Dendritic cells may, of course, be engineered to express specific cell-surface receptors or ligands that are not commonly found on dendritic cells *in vivo* or *ex vivo*, and such modified dendritic cells are contemplated by the present invention. As an alternative to dendritic cells, secreted vesicles antigen-loaded dendritic cells (called exosomes) may be used within a vaccine, or immunogenic composition (*see* Zitvogel *et al.*, *Nature Med.* 4:594-600, 1998).

Dendritic cells and progenitors may be obtained from peripheral blood, bone marrow, tumor-infiltrating cells, peritumoral tissues-infiltrating cells, lymph nodes, spleen,

skin, umbilical cord blood or any other suitable tissue or fluid. For example, dendritic cells may be differentiated *ex vivo* by adding a combination of cytokines such as GM-CSF, IL-4, IL-13 and/or TNF α to cultures of monocytes harvested from peripheral blood. Alternatively, CD34 positive cells harvested from peripheral blood, umbilical cord blood or
 5 bone marrow may be differentiated into dendritic cells by adding to the culture medium combinations of GM-CSF, IL-3, TNF α , CD40 ligand, LPS, flt3 ligand and/or other compound(s) that induce differentiation, maturation and proliferation of dendritic cells.

Dendritic cells are conveniently categorized as "immature" and "mature" cells, which allows a simple way to discriminate between two well characterized
 10 phenotypes. However, this nomenclature should not be construed to exclude all possible intermediate stages of differentiation. Immature dendritic cells are characterized as APC with a high capacity for antigen uptake and processing, which correlates with the high expression of Fc γ receptor and mannose receptor. The mature phenotype is typically characterized by a lower expression of these markers, but a high expression of cell surface
 15 molecules responsible for T cell activation such as class I and class II MHC, adhesion molecules (*e.g.*, CD54 and CD11) and costimulatory molecules (*e.g.*, CD40, CD80, CD86 and 4-1BB).

APCs may generally be transfected with a polynucleotide encoding a lung tumor protein (or portion or other variant thereof) such that the lung tumor polypeptide, or
 20 an immunogenic portion thereof, is expressed on the cell surface. Such transfection may take place *ex vivo*, and a composition comprising such transfected cells may then be used for therapeutic purposes, as described herein. Alternatively, a gene delivery vehicle that targets a dendritic or other antigen presenting cell may be administered to a patient, resulting in transfection that occurs *in vivo*. *In vivo* and *ex vivo* transfection of dendritic
 25 cells, for example, may generally be performed using any methods known in the art, such as those described in WO 97/24447, or the gene gun approach described by Mahvi *et al.*, *Immunology and cell Biology* 75:456-460, 1997. Antigen loading of dendritic cells may be achieved by incubating dendritic cells or progenitor cells with the lung tumor polypeptide, DNA (naked or within a plasmid vector) or RNA; or with antigen-expressing recombinant

bacterium or viruses (*e.g.*, vaccinia, fowlpox, adenovirus or lentivirus vectors). Prior to loading, the polypeptide may be covalently conjugated to an immunological partner that provides T cell help (*e.g.*, a carrier molecule). Alternatively, a dendritic cell may be pulsed with a non-conjugated immunological partner, separately or in the presence of the polypeptide.

Immunogenic compositions and pharmaceutical compositions may be presented in unit-dose or multi-dose containers, such as sealed ampoules or vials. Such containers are preferably hermetically sealed to preserve sterility of the formulation until use. In general, formulations may be stored as suspensions, solutions or emulsions in oily or aqueous vehicles. Alternatively, an immunogenic or pharmaceutical composition may be stored in a freeze-dried condition requiring only the addition of a sterile liquid carrier immediately prior to use.

CANCER THERAPY

In further aspects of the present invention, the compositions described herein may be used for immunotherapy of cancer, such as lung cancer. Within such methods, compositions are typically administered to a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human. A patient may or may not be afflicted with cancer. Accordingly, the above pharmaceutical compositions and immunogenic compositions may be used to prevent the development of a cancer or to treat a patient afflicted with a cancer. A cancer may be diagnosed using criteria generally accepted in the art, including the presence of a malignant tumor. Pharmaceutical compositions and immunogenic compositions may be administered either prior to or following surgical removal of primary tumors and/or treatment such as administration of radiotherapy or conventional chemotherapeutic drugs. Administration may be by any suitable method, including administration by intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal, intradermal, anal, vaginal, topical and oral routes.

Within certain embodiments, immunotherapy may be active immunotherapy, in which treatment relies on the *in vivo* stimulation of the endogenous host

immune system to react against tumors with the administration of immune response-modifying agents (such as polypeptides and polynucleotides as provided herein).

Within other embodiments, immunotherapy may be passive immunotherapy, in which treatment involves the delivery of agents with established tumor-immune reactivity (such as effector cells or antibodies) that can directly or indirectly
5 mediate antitumor effects and does not necessarily depend on an intact host immune system. Examples of effector cells include T cells as discussed above, T lymphocytes (such as CD8⁺ cytotoxic T lymphocytes and CD4⁺ T-helper tumor-infiltrating lymphocytes), killer cells (such as Natural Killer cells and lymphokine-activated killer
10 cells), B cells and antigen-presenting cells (such as dendritic cells and macrophages) expressing a polypeptide provided herein. T cell receptors and antibody receptors specific for the polypeptides recited herein may be cloned, expressed and transferred into other vectors or effector cells for adoptive immunotherapy. The polypeptides provided herein may also be used to generate antibodies or anti-idiotypic antibodies (as described above
15 and in U.S. Patent No. 4,918,164) for passive immunotherapy.

Effector cells may generally be obtained in sufficient quantities for adoptive immunotherapy by growth *in vitro*, as described herein. Culture conditions for expanding single antigen-specific effector cells to several billion in number with retention of antigen recognition *in vivo* are well known in the art. Such *in vitro* culture conditions typically use
20 intermittent stimulation with antigen, often in the presence of cytokines (such as IL-2) and non-dividing feeder cells. As noted above, immunoreactive polypeptides as provided herein may be used to rapidly expand antigen-specific T cell cultures in order to generate a sufficient number of cells for immunotherapy. In particular, antigen-presenting cells, such as dendritic, macrophage, monocyte, fibroblast and/or B cells, may be pulsed with
25 immunoreactive polypeptides or transfected with one or more polynucleotides using standard techniques well known in the art. For example, antigen-presenting cells can be transfected with a polynucleotide having a promoter appropriate for increasing expression in a recombinant virus or other expression system. Cultured effector cells for use in therapy must be able to grow and distribute widely, and to survive long term *in vivo*.

Studies have shown that cultured effector cells can be induced to grow *in vivo* and to survive long term in substantial numbers by repeated stimulation with antigen supplemented with IL-2 (*see, for example, Cheever et al., Immunological Reviews 157:177, 1997*).

- 5 Alternatively, a vector expressing a polypeptide recited herein may be introduced into antigen presenting cells taken from a patient and clonally propagated *ex vivo* for transplant back into the same patient. Transfected cells may be reintroduced into the patient using any means known in the art, preferably in sterile form by intravenous, intracavitary, intraperitoneal or intratumor administration.
- 10 Routes and frequency of administration of the therapeutic compositions described herein, as well as dosage, will vary from individual to individual, and may be readily established using standard techniques. In general, the pharmaceutical compositions and immunogenic compositions may be administered by injection (*e.g.*, intracutaneous, intramuscular, intravenous or subcutaneous), intranasally (*e.g.*, by aspiration) or orally.
- 15 Preferably, between 1 and 10 doses may be administered over a 52 week period. Preferably, 6 doses are administered, at intervals of 1 month, and booster vaccinations may be given periodically thereafter. Alternate protocols may be appropriate for individual patients. A suitable dose is an amount of a compound that, when administered as described above, is capable of promoting an anti-tumor immune response, and is at least 10-50%
- 20 above the basal (*i.e.*, untreated) level. Such response can be monitored by measuring the anti-tumor antibodies in a patient or by vaccine-dependent generation of cytolytic effector cells capable of killing the patient's tumor cells *in vitro*. Such vaccines, or immunogenic compositions, should also be capable of causing an immune response that leads to an improved clinical outcome (*e.g.*, more frequent remissions, complete or partial or longer
- 25 disease-free survival) in vaccinated patients as compared to non-vaccinated patients. In general, for compositions comprising one or more polypeptides, the amount of each polypeptide present in a dose ranges from about 25 μ g to 5 mg per kg of host. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 mL to about 5 mL.

In general, an appropriate dosage and treatment regimen provides the active compound(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit. Such a response can be monitored by establishing an improved clinical outcome (*e.g.*, more frequent remissions, complete or partial, or longer disease-free survival) in treated patients as compared to non-treated patients. Increases in preexisting immune responses to a lung tumor protein generally correlate with an improved clinical outcome. Such immune responses may generally be evaluated using standard proliferation, cytotoxicity or cytokine assays, which may be performed using samples obtained from a patient before and after treatment.

10 CANCER DETECTION AND DIAGNOSIS

In general, a cancer may be detected in a patient based on the presence of one or more lung tumor proteins and/or polynucleotides encoding such proteins in a biological sample (for example, blood, sera, sputum urine and/or tumor biopsies) obtained from the patient. In other words, such proteins may be used as markers to indicate the presence or absence of a cancer such as lung cancer. In addition, such proteins may be useful for the detection of other cancers. The binding agents provided herein generally permit detection of the level of antigen that binds to the agent in the biological sample. Polynucleotide primers and probes may be used to detect the level of mRNA encoding a tumor protein, which is also indicative of the presence or absence of a cancer. In general, lung tumor sequence should be present at a level that is at least three fold higher in tumor tissue than in normal tissue

There are a variety of assay formats known to those of ordinary skill in the art for using a binding agent to detect polypeptide markers in a sample. *See, e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In general, the presence or absence of a cancer in a patient may be determined by (a) contacting a biological sample obtained from a patient with a binding agent; (b) detecting in the sample a level of polypeptide that binds to the binding agent; and (c) comparing the level of polypeptide with a predetermined cut-off value.

In a preferred embodiment, the assay involves the use of binding agent immobilized on a solid support to bind to and remove the polypeptide from the remainder of the sample. The bound polypeptide may then be detected using a detection reagent that contains a reporter group and specifically binds to the binding agent/polypeptide complex.

- 5 Such detection reagents may comprise, for example, a binding agent that specifically binds to the polypeptide or an antibody or other agent that specifically binds to the binding agent, such as an anti-immunoglobulin, protein G, protein A or a lectin. Alternatively, a competitive assay may be utilized, in which a polypeptide is labeled with a reporter group and allowed to bind to the immobilized binding agent after incubation of the binding agent
- 10 with the sample. The extent to which components of the sample inhibit the binding of the labeled polypeptide to the binding agent is indicative of the reactivity of the sample with the immobilized binding agent. Suitable polypeptides for use within such assays include full length lung tumor proteins and portions thereof to which the binding agent binds, as described above.

- 15 The solid support may be any material known to those of ordinary skill in the art to which the tumor protein may be attached. For example, the solid support may be a test well in a microtiter plate or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic
- 20 particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681. The binding agent may be immobilized on the solid support using a variety of techniques known to those of skill in the art, which are amply described in the patent and scientific literature. In the context of the present invention, the term "immobilization" refers to both noncovalent association, such as adsorption, and covalent attachment (which
- 25 may be a direct linkage between the agent and functional groups on the support or may be a linkage by way of a cross-linking agent). Immobilization by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the binding agent, in a suitable buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1

hour and about 1 day. In general, contacting a well of a plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of binding agent ranging from about 10 ng to about 10 μ g, and preferably about 100 ng to about 1 μ g, is sufficient to immobilize an adequate amount of binding agent.

- 5 Covalent attachment of binding agent to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the binding agent. For example, the binding agent may be covalently attached to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group
10 on the support with an amine and an active hydrogen on the binding partner (*see, e.g.*, Pierce Immunotechnology Catalog and Handbook, 1991, at A12-A13).

- In certain embodiments, the assay is a two-antibody sandwich assay. This assay may be performed by first contacting an antibody that has been immobilized on a solid support, commonly the well of a microtiter plate, with the sample, such that
15 polypeptides within the sample are allowed to bind to the immobilized antibody. Unbound sample is then removed from the immobilized polypeptide-antibody complexes and a detection reagent (preferably a second antibody capable of binding to a different site on the polypeptide) containing a reporter group is added. The amount of detection reagent that remains bound to the solid support is then determined using a method appropriate for the
20 specific reporter group.

- More specifically, once the antibody is immobilized on the support as described above, the remaining protein binding sites on the support are typically blocked. Any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin or Tween 20TM (Sigma Chemical Co., St. Louis, MO). The immobilized
25 antibody is then incubated with the sample, and polypeptide is allowed to bind to the antibody. The sample may be diluted with a suitable diluent, such as phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact time (*i.e.*, incubation time) is a period of time that is sufficient to detect the presence of polypeptide within a sample obtained from an individual with lung cancer. Preferably, the contact time is

sufficient to achieve a level of binding that is at least about 95% of that achieved at equilibrium between bound and unbound polypeptide. Those of ordinary skill in the art will recognize that the time necessary to achieve equilibrium may be readily determined by assaying the level of binding that occurs over a period of time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

Unbound sample may then be removed by washing the solid support with an appropriate buffer, such as PBS containing 0.1% Tween 20™. The second antibody, which contains a reporter group, may then be added to the solid support. Preferred reporter groups include those groups recited above.

The detection reagent is then incubated with the immobilized antibody-polypeptide complex for an amount of time sufficient to detect the bound polypeptide. An appropriate amount of time may generally be determined by assaying the level of binding that occurs over a period of time. Unbound detection reagent is then removed and bound detection reagent is detected using the reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products.

To determine the presence or absence of a cancer, such as lung cancer, the signal detected from the reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value. In one preferred embodiment, the cut-off value for the detection of a cancer is the average mean signal obtained when the immobilized antibody is incubated with samples from patients without the cancer. In general, a sample generating a signal that is three standard deviations above the predetermined cut-off value is considered positive for the cancer. In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve,

according to the method of Sackett *et al.*, *Clinical Epidemiology: A Basic Science for Clinical Medicine*, Little Brown and Co., 1985, p. 106-7. Briefly, in this embodiment, the cut-off value may be determined from a plot of pairs of true positive rates (*i.e.*, sensitivity) and false positive rates (100%-specificity) that correspond to each possible cut-off value
 5 for the diagnostic test result. The cut-off value on the plot that is the closest to the upper left-hand corner (*i.e.*, the value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut-off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the
 10 false negative rate. In general, a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for a cancer.

In a related embodiment, the assay is performed in a flow-through or strip test format, wherein the binding agent is immobilized on a membrane, such as nitrocellulose. In the flow-through test, polypeptides within the sample bind to the
 15 immobilized binding agent as the sample passes through the membrane. A second, labeled binding agent then binds to the binding agent-polypeptide complex as a solution containing the second binding agent flows through the membrane. The detection of bound second binding agent may then be performed as described above. In the strip test format, one end of the membrane to which binding agent is bound is immersed in a solution containing the
 20 sample. The sample migrates along the membrane through a region containing second binding agent and to the area of immobilized binding agent. Concentration of second binding agent at the area of immobilized antibody indicates the presence of a cancer. Typically, the concentration of second binding agent at that site generates a pattern, such as a line, that can be read visually. The absence of such a pattern indicates a negative result.
 25 In general, the amount of binding agent immobilized on the membrane is selected to generate a visually discernible pattern when the biological sample contains a level of polypeptide that would be sufficient to generate a positive signal in the two-antibody sandwich assay, in the format discussed above. Preferred binding agents for use in such assays are antibodies and antigen-binding fragments thereof. Preferably, the amount of

antibody immobilized on the membrane ranges from about 25 ng to about 1 μ g, and more preferably from about 50 ng to about 500 ng. Such tests can typically be performed with a very small amount of biological sample.

Of course, numerous other assay protocols exist that are suitable for use
 5 with the tumor proteins or binding agents of the present invention. The above descriptions are intended to be exemplary only. For example, it will be apparent to those of ordinary skill in the art that the above protocols may be readily modified to use lung tumor polypeptides to detect antibodies that bind to such polypeptides in a biological sample. The detection of such lung tumor protein specific antibodies may correlate with the
 10 presence of a cancer.

A cancer may also, or alternatively, be detected based on the presence of T cells that specifically react with a lung tumor protein in a biological sample. Within certain methods, a biological sample comprising CD4⁺ and/or CD8⁺ T cells isolated from a patient is incubated with a lung tumor polypeptide, a polynucleotide encoding such a polypeptide
 15 and/or an APC that expresses at least an immunogenic portion of such a polypeptide, and the presence or absence of specific activation of the T cells is detected. Suitable biological samples include, but are not limited to, isolated T cells. For example, T cells may be isolated from a patient by routine techniques (such as by Ficoll/Hypaque density gradient centrifugation of peripheral blood lymphocytes). T cells may be incubated *in vitro* for 2-9
 20 days (typically 4 days) at 37°C with polypeptide (*e.g.*, 5 - 25 μ g/ml). It may be desirable to incubate another aliquot of a T cell sample in the absence of lung tumor polypeptide to serve as a control. For CD4⁺ T cells, activation is preferably detected by evaluating proliferation of the T cells. For CD8⁺ T cells, activation is preferably detected by evaluating cytolytic activity. A level of proliferation that is at least two fold greater and/or
 25 a level of cytolytic activity that is at least 20% greater than in disease-free patients indicates the presence of a cancer in the patient.

As noted above, a cancer may also, or alternatively, be detected based on the level of mRNA encoding a lung tumor protein in a biological sample. For example, at least two oligonucleotide primers may be employed in a polymerase chain reaction (PCR) based

assay to amplify a portion of a lung tumor cDNA derived from a biological sample, wherein at least one of the oligonucleotide primers is specific for (*i.e.*, hybridizes to) a polynucleotide encoding the lung tumor protein. The amplified cDNA is then separated and detected using techniques well known in the art, such as gel electrophoresis. Similarly, 5 oligonucleotide probes that specifically hybridize to a polynucleotide encoding a lung tumor protein may be used in a hybridization assay to detect the presence of polynucleotide encoding the tumor protein in a biological sample.

To permit hybridization under assay conditions, oligonucleotide primers and probes should comprise an oligonucleotide sequence that has at least about 60%, preferably 10 at least about 75% and more preferably at least about 90%, identity to a portion of a polynucleotide encoding a lung tumor protein that is at least 10 nucleotides, and preferably at least 20 nucleotides, in length. Preferably, oligonucleotide primers and/or probes hybridize to a polynucleotide encoding a polypeptide described herein under moderately stringent conditions, as defined above. Oligonucleotide primers and/or probes which may 15 be usefully employed in the diagnostic methods described herein preferably are at least 10-40 nucleotides in length. In a preferred embodiment, the oligonucleotide primers comprise at least 10 contiguous nucleotides, more preferably at least 15 contiguous nucleotides, of a DNA molecule having a sequence recited in SEQ ID NO: 1, 11-13, 15, 20, 23-27, 29, 30, 33, 34, 39, 41, 43-46, 51, 52, 57, 58, 60, 62, 65-67, 69-71, 74, 76, 79, 80, 84, 86, 89-92, 95, 20 97, 98, 101, 110, 111, 113-119, 121-128, 130-134, 136, 138, 139, 141, 143, 146-151, 153, 154, 157-160, 162-164, 167-178, 180, 181, 183, 186-190, 192, 193, 195-220, 224, 226-231, 234, 236, 237, 240, 241, 244-246, 248, 254, 255, 261, 262, 266, 270, 275, 280, 282, 283, 288, 289, 290, 292, 295, 301, 303, 304, 309, 311, 341-782, 784, 785, 790, 792, 794, 796, 800-804, 807, 808, 810-826, 828-1664, 1669 or 1676. Techniques for both PCR 25 based assays and hybridization assays are well known in the art (*see*, for example, Mullis *et al.*, *Cold Spring Harbor Symp. Quant. Biol.*, 51:263, 1987; Erlich ed., *PCR Technology*, Stockton Press, NY, 1989).

One preferred assay employs RT-PCR, in which PCR is applied in conjunction with reverse transcription. Typically, RNA is extracted from a biological

sample, such as biopsy tissue, and is reverse transcribed to produce cDNA molecules. PCR amplification using at least one specific primer generates a cDNA molecule, which may be separated and visualized using, for example, gel electrophoresis. Amplification may be performed on biological samples taken from a test patient and from an individual who is not afflicted with a cancer. The amplification reaction may be performed on several dilutions of cDNA spanning two orders of magnitude. A two-fold or greater increase in expression in several dilutions of the test patient sample as compared to the same dilutions of the non-cancerous sample is typically considered positive.

In another embodiment, the compositions described herein may be used as markers for the progression of cancer. In this embodiment, assays as described above for the diagnosis of a cancer may be performed over time, and the change in the level of reactive polypeptide(s) or polynucleotide(s) evaluated. For example, the assays may be performed every 24-72 hours for a period of 6 months to 1 year, and thereafter performed as needed. In general, a cancer is progressing in those patients in whom the level of polypeptide or polynucleotide detected increases over time. In contrast, the cancer is not progressing when the level of reactive polypeptide or polynucleotide either remains constant or decreases with time.

Certain *in vivo* diagnostic assays may be performed directly on a tumor. One such assay involves contacting tumor cells with a binding agent. The bound binding agent may then be detected directly or indirectly via a reporter group. Such binding agents may also be used in histological applications. Alternatively, polynucleotide probes may be used within such applications.

As noted above, to improve sensitivity, multiple lung tumor protein markers may be assayed within a given sample. It will be apparent that binding agents specific for different proteins provided herein may be combined within a single assay. Further, multiple primers or probes may be used concurrently. The selection of tumor protein markers may be based on routine experiments to determine combinations that results in optimal sensitivity. In addition, or alternatively, assays for tumor proteins provided herein may be combined with assays for other known tumor antigens.

DIAGNOSTIC KITS

The present invention further provides kits for use within any of the above diagnostic methods. Such kits typically comprise two or more components necessary for performing a diagnostic assay. Components may be compounds, reagents, containers
5 and/or equipment. For example, one container within a kit may contain a monoclonal antibody or fragment thereof that specifically binds to a lung tumor protein. Such antibodies or fragments may be provided attached to a support material, as described above. One or more additional containers may enclose elements, such as reagents or buffers, to be used in the assay. Such kits may also, or alternatively, contain a detection
10 reagent as described above that contains a reporter group suitable for direct or indirect detection of antibody binding.

Alternatively, a kit may be designed to detect the level of mRNA encoding a lung tumor protein in a biological sample. Such kits generally comprise at least one oligonucleotide probe or primer, as described above, that hybridizes to a polynucleotide
15 encoding a lung tumor protein. Such an oligonucleotide may be used, for example, within a PCR or hybridization assay. Additional components that may be present within such kits include a second oligonucleotide and/or a diagnostic reagent or container to facilitate the detection of a polynucleotide encoding a lung tumor protein.

The following Examples are offered by way of illustration and not by way
20 of limitation.

EXAMPLE 1IDENTIFICATION AND CHARACTERIZATION OF LUNG
TUMOR PROTEIN cDNAS

5 This Example illustrates the identification of cDNA molecules encoding lung tumor proteins.

A. Isolation of cDNA Sequences from Lung Adenocarcinoma Libraries using
Conventional cDNA Library Subtraction

 A human lung adenocarcinoma cDNA expression library was constructed
10 from poly A⁺ RNA from patient tissues (# 40031486) using a Superscript Plasmid System for cDNA Synthesis and Plasmid Cloning kit (BRL Life Technologies, Gaithersburg, MD) following the manufacturer's protocol. Specifically, lung carcinoma tissues were homogenized with polytron (Kinematica, Switzerland) and total RNA was extracted using Trizol reagent (BRL Life Technologies) as directed by the manufacturer. The poly A⁺
15 RNA was then purified using an oligo dT cellulose column as described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989. First-strand cDNA was synthesized using the NotI/Oligo-dT18 primer. Double-stranded cDNA was synthesized, ligated with BstXI/EcoRI adaptors (Invitrogen, San Diego, CA) and digested with NotI. Following size fractionation with cDNA size
20 fractionation columns (BRL Life Technologies), the cDNA was ligated into the BstXI/NotI site of pcDNA3.1 (Invitrogen) and transformed into ElectroMax *E. coli* DH10B cells (BRL Life Technologies) by electroporation. A total of 3 x 10⁶ independent colonies were generated.

 Using the same procedure, a normal human cDNA expression library was
25 prepared from a panel of normal tissue specimens, including lung, liver, pancreas, skin, kidney, brain and resting PBMC.

 cDNA library subtraction was performed using the above lung adenocarcinoma and normal tissue cDNA libraries, as described by Hara *et al.* (*Blood*,

84:189-199, 1994) with some modifications. Specifically, a lung adenocarcinoma-specific subtracted cDNA library was generated as follows. The normal tissue cDNA library (80 μ g) was digested with BamHI and XhoI, followed by a filling-in reaction with DNA polymerase Klenow fragment. After phenol-chloroform extraction and ethanol precipitation, the DNA was dissolved in 133 μ l of H₂O, heat-denatured and mixed with 133 μ l (133 μ g) of Photoprobe biotin (Vector Laboratories, Burlingame, CA). As recommended by the manufacturer, the resulting mixture was irradiated with a 270 W sunlamp on ice for 20 minutes. Additional Photoprobe biotin (67 μ l) was added and the biotinylation reaction was repeated. After extraction with butanol five times, the DNA was ethanol-precipitated and dissolved in 23 μ l H₂O. The resulting DNA, plus other highly redundant cDNA clones that were frequently recovered in previous lung subtractions formed the driver DNA.

To form the tracer DNA, 10 μ g lung adenocarcinoma cDNA library was digested with NotI and SpeI, phenol chloroform extracted and passed through Chroma spin-400 columns (Clontech, Palo Alto, CA). Typically, 5 μ g of cDNA was recovered after the sizing column. Following ethanol precipitation, the tracer DNA was dissolved in 5 μ l H₂O. Tracer DNA was mixed with 15 μ l driver DNA and 20 μ l of 2 x hybridization buffer (1.5 M NaCl/10 mM EDTA/50 mM HEPES pH 7.5/0.2% sodium dodecyl sulfate), overlaid with mineral oil, and heat-denatured completely. The sample was immediately transferred into a 68 °C water bath and incubated for 20 hours (long hybridization [LH]). The reaction mixture was then subjected to a streptavidin treatment followed by phenol/chloroform extraction. This process was repeated three more times. Subtracted DNA was precipitated, dissolved in 12 μ l H₂O, mixed with 8 μ l driver DNA and 20 μ l of 2 x hybridization buffer, and subjected to a hybridization at 68°C for 2 hours (short hybridization [SH]). After removal of biotinylated double-stranded DNA, subtracted cDNA was ligated into NotI/SpeI site of chloramphenicol resistant pBCSK⁺ (Stratagene, La Jolla, CA) and transformed into ElectroMax *E. coli* DH10B cells by electroporation to generate a lung adenocarcinoma specific subtracted cDNA library, referred to as LAT-S1

Similarly, LAT-S2 was generated by including 23 genes that were over-expressed in the tracer as additional drivers.

A second human lung adenocarcinoma cDNA expression library was constructed using adenocarcinoma tissue from a second patient (# 86-66) and used to
5 prepare a second lung adenocarcinoma-specific subtracted cDNA library (referred to as LAT2-S2), as described above, using the same panel of normal tissues and the additional genes over-expressed in LAT-S1.

A third human metastatic lung adenocarcinoma library was constructed from a pool of two lung pleural effusions with lung and gastric adenocarcinoma origins.
10 The subtracted cDNA library, Mets-sub2 was generated as described above using the same panel of normal tissues. However, the Mets-sub3 subtracted library was constructed by including 51 additional genes as drivers. These 51 genes were recovered in Mets-sub2, representing over-expressed housekeeping genes in the testers. As a result, Mets-sub3 is more complexed and normalized.

A total of 16 cDNA fragments isolated from LAT-S1, 585 cDNA fragments isolated from LAT-S2, 568 cDNA clones from LAT2-S2, 15 cDNA clones from Mets-sub2 and 343 cDNA clones from Mets-sub3, described above, were colony PCR amplified and their mRNA expression levels in lung tumor, normal lung, and various other normal and tumor tissues were determined using microarray technology (Incyte, Palo Alto, CA).
15 Briefly, the PCR amplification products were dotted onto slides in an array format, with each product occupying a unique location in the array. mRNA was extracted from the tissue sample to be tested, reverse transcribed, and fluorescent-labeled cDNA probes were generated. The microarrays were probed with the labeled cDNA probes, the slides scanned and fluorescence intensity was measured. This intensity correlates with the hybridization
20 intensity. Seventy-three non-redundant cDNA clones, of which 42 were found to be unique, showed over-expression in lung tumors, with expression in normal tissues tested (lung, skin, lymph node, colon, liver, pancreas, breast, heart, bone marrow, large intestine, kidney, stomach, brain, small intestine, bladder and salivary gland) being either undetectable, or at significantly lower levels compared to lung adenocarcinoma tumors.

These clones were further characterized by DNA sequencing with a Perkin Elmer/Applied Biosystems Division Automated Sequencer Model 373A and/or Model 377 (Foster City, CA).

The sequences were compared to known sequences in the gene bank using the EMBL GenBank databases (release 96). No significant homologies were found to the sequence provided in SEQ ID NO: 67, with no apparent homology to previously identified expressed sequence tags (ESTs). The sequences of SEQ ID NO: 60, 62, 65, 66, 69-71, 74, 76, 79, 80, 84, 86, 89-92, 95, 97 and 98 were found to show some homology to previously identified expressed sequence tags (ESTs). The cDNA sequences of SEQ ID NO: 59, 61, 63, 64, 67, 68, 72, 73, 75, 77, 78, 81-83, 85, 87, 88, 93, 94, 96, 99 and 100 showed homology to previously identified genes. The full-length cDNA sequences for the clones of SEQ ID NO: 96 and 100 are provided in SEQ ID NO: 316 and 318, respectively. The amino acid sequences for the clones of SEQ ID NO: 59, 61, 63, 64, 68, 73, 82, 83, 94, 96 and 100 are provided in SEQ ID NO: 331, 328, 329, 332, 327, 333, 330, 326, 325, 324 and 335, respectively. A predicted amino acid sequence encoded by the sequence of SEQ ID NO: 69 (referred to as L552S) is provided in SEQ ID NO: 786.

Further studies led to the isolation of an extended cDNA sequence, and open reading frame, for L552S (SEQ ID NO: 790). The predicted amino acid sequence encoded by the cDNA sequence of SEQ ID NO: 790 is provided in SEQ ID NO: 791. The determined cDNA sequence of an isoform of L552S is provided in SEQ ID NO: 792, with the corresponding predicted amino acid sequence being provided in SEQ ID NO: 793. Subsequent studies led to the isolation of the full-length cDNA sequence of L552S (SEQ ID NO: 808). The corresponding amino acid sequence is provided in SEQ ID NO: 809. No homologies were found to the protein sequence of L552S. However, nucleotides 533-769 of the full-length cDNA sequence were found to show homology to a previously identified DNA sequence.

Full-length cloning efforts on L552S led to the isolation of three additional cDNA sequences (SEQ ID NO: 810-812) from a metastatic lung adenocarcinoma library. The sequence of SEQ ID NO: 810 was found to show some homology to previously

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identified human DNA sequences. The sequence of SEQ ID NO: 811 was found to show some homology to a previously identified DNA sequence. The sequence of SEQ ID NO: 812 was found to show some homology to previously identified ESTs.

The gene of SEQ ID NO: 84 (referred to as L551S) was determined by real-time RT-PCR analysis to be over-expressed in 2/9 primary adenocarcinomas and to be expressed at lower levels in 2/2 metastatic adenocarcinomas and 1/2 squamous cell carcinomas. No expression was observed in normal tissues, with the exception of very low expression in normal stomach. Further studies on L551S led to the isolation of the 5' and 3' cDNA consensus sequences provided in SEQ ID NO: 801 and 802, respectively. The L551S 5' sequence was found to show some homology to the previously identified gene STY8 (cDNA sequence provided in SEQ ID NO: 803; corresponding amino acid sequence provided in SEQ ID NO: 805), which is a mitogen activated protein kinase phosphatase. However, no significant homologies were found to the 3' sequence of L551S. Subsequently, an extended cDNA sequence for L551S was isolated (SEQ ID NO: 804). The corresponding amino acid sequence is provided in SEQ ID NO: 806. Further studies led to the isolation of two independent full-length clones for L551S (referred to as 54298 and 54305). These two clones have five nucleotide differences compared to the STY8 DNA sequence. Two of these differences are single nucleotide polymorphisms which do not effect the encoded amino acid sequences. The other three nucleotide differences are consistent between the two L551S clones but lead to encoded amino acid sequences that are different from the STY8 protein sequence. The determined cDNA sequences for the L551S full-length clones 54305 and 54298 are provided in SEQ ID NO: 825 and 826, respectively, with the amino acid sequence for L551S being provided in SEQ ID NO: 827.

B. Isolation of cDNA Sequences from Lung Adenocarcinoma Libraries using PCR-Based cDNA Library Subtraction

cDNA clones from a PCR-based subtraction library, containing cDNA from a pool of two human lung primary adenocarcinomas subtracted against a pool of nine normal human tissue cDNAs including skin, colon, lung, esophagus, brain, kidney, spleen,

pancreas and liver, (Clontech, Palo Alto, CA) were derived and submitted to a first round of PCR amplification. This library (referred to as ALT-1) was subjected to a second round of PCR amplification, following the manufacturer's protocol. The expression levels of 760 cDNA clones in lung tumor, normal lung, and various other normal and tumor tissues, were examined using microarray technology as described above. A total of 118 clones, of which 55 were unique, were found to be over-expressed in lung tumor tissue, with expression in normal tissues tested (lung, skin, lymph node, colon, liver, pancreas, breast, heart, bone marrow, large intestine, kidney, stomach, brain, small intestine, bladder and salivary gland) being either undetectable, or at significantly lower levels. The sequences were compared to known sequences in the gene bank using the EMBL and GenBank databases (release 96). No significant homologies (including ESTs) were found to the sequence provided in SEQ ID NO: 44. The sequences of SEQ ID NO: 1, 11, 13, 15, 20, 23-27, 29, 30, 33, 34, 39, 41, 43, 45, 46, 51 and 57 were found to show some homology to previously identified expressed sequence tags (ESTs). The cDNA sequences of SEQ ID NO: 2-10, 12, 14, 16-19, 21, 22, 28, 31, 32, 35-38, 40, 42, 44, 47-50, 52-56 and 58 showed homology to previously identified genes. The full-length cDNA sequences for the clones of SEQ ID NO: 18, 22, 31, 35, 36 and 42 are provided in SEQ ID NO: 320, 319, 323, 321, 317, 321 and 322, respectively, with the corresponding amino acid sequences being provided in SEQ ID NO: 337, 336, 340, 338, 334, and 339, respectively.

Further studies led to the isolation of an extended cDNA sequence for the clone of SEQ ID NO: 33 (referred to as L801P). This extended cDNA sequence (provided in SEQ ID NO: 796), was found to contain three potential open reading frames (ORFs). The predicted amino acid sequences encoded by these three ORFs are provided in SEQ ID NO: 797-799, respectively. Additional full-length cloning efforts led to still further extended cDNA sequence for L801P, set forth in SEQ ID NO:1669, in addition to five potential open reading frames (ORFs 4-9; SEQ ID NOs: 1670-1675, respectively) encoded by the extended cDNA sequence. Moreover, L801P was mapped to chromosomal region 20p13 and a 137 amino acid ORF from this genomic region was identified that corresponds to ORF4 (SEQ ID NO: 1670), suggesting that this is likely an authentic ORF for L801P.

We have also found that L801P expression is detected in a number of other tumor types, including breast, prostate, ovarian and colon tumors, and thus may have diagnostic and/or therapeutic utility in these cancer types as well.

The predicted amino acid sequence encoded by the sequence of SEQ ID NO: 46 (referred to as L840P) is provided in SEQ ID NO: 787. An extended cDNA sequence for L840P, which was determined to include an open reading frame, is provided in SEQ ID NO: 794. The predicted amino acid sequence encoded by the cDNA sequence of SEQ ID NO: 794 is provided in SEQ ID NO: 795. The full-length cDNA sequence for the clone of SEQ ID NO: 54 (referred to as L548S) is provided in SEQ ID NO: 788, with the corresponding amino acid sequence being provided in SEQ ID NO: 789.

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Further studies on L773P (SEQ ID NO: 58) resulted in the isolation of the extended consensus cDNA sequence provided in SEQ ID NO: 807.

Additional lung adenocarcinoma cDNA clones were isolated as follows. A cDNA library was prepared from a pool of two lung adenocarcinomas and subtracted
5 against cDNA from a panel of normal tissues including lung, brain, liver, kidney, pancreas, skin, heart and spleen. The subtraction was performed using a PCR-based protocol (Clontech), which was modified to generate larger fragments. Within this protocol, tester and driver double stranded cDNA were separately digested with five restriction enzymes that recognize six-nucleotide restriction sites (MluI, MscI, PvuII, SalI and StuI). This
10 digestion resulted in an average cDNA size of 600 bp, rather than the average size of 300 bp that results from digestion with RsaI according to the Clontech protocol. The ends of the restriction digested tester cDNA were filled in to generate blunt ends for adapter ligation. This modification did not affect the subtraction efficiency. Two tester populations were then created with different adapters, and the driver library remained
15 without adapters. The tester and driver libraries were then hybridized using excess driver cDNA. In the first hybridization step, driver was separately hybridized with each of the two tester cDNA populations. This resulted in populations of (a) unhybridized tester cDNAs, (b) tester cDNAs hybridized to other tester cDNAs, (c) tester cDNAs hybridized to driver cDNAs and (d) unhybridized driver cDNAs. The two separate hybridization
20 reactions were then combined, and rehybridized in the presence of additional denatured driver cDNA. Following this second hybridization, in addition to populations (a) through (d), a fifth population (e) was generated in which tester cDNA with one adapter hybridized to tester cDNA with the second adapter. Accordingly, the second hybridization step resulted in enrichment of differentially expressed sequences which could be used as
25 templates for PCR amplification with adaptor-specific primers.

The ends were then filled in, and PCR amplification was performed using adaptor-specific primers. Only population (e), which contained tester cDNA that did not hybridize to driver cDNA, was amplified exponentially. A second PCR amplification step

was then performed, to reduce background and further enrich differentially expressed sequences.

Fifty-seven cDNA clones were isolated from the subtracted library (referred to as LAP1) and sequenced. The determined cDNA sequences for 16 of these clones are provided in SEQ ID NO: 101-116. The sequences of SEQ ID NO: 101 and 114 showed no significant homologies to previously identified sequences. The sequences of SEQ ID NO: 102-109 and 112 showed some similarity to previously identified sequences, while the sequences of SEQ ID NO: 113, 115 and 116 showed some similarity to previously isolated ESTs.

An additional 502 clones analyzed from the LAP1 library were sequenced and the determined cDNA sequences are shown in SEQ ID NO:828-1239 and 1564-1653.

C. Isolation of cDNA Sequences from Small Cell Lung Carcinoma Libraries using PCR-Based cDNA Library Subtraction

A subtracted cDNA library for small cell lung carcinoma (referred to as SCL1) was prepared using essentially the modified PCR-based subtraction process described above. cDNA from small cell lung carcinoma was subtracted against cDNA from a panel of normal tissues, including normal lung, brain, kidney, liver, pancreas, skin, heart, lymph node and spleen. Both tester and driver poly A+ RNA were initially amplified using SMART PCR cDNA synthesis kit (Clontech, Palo Alto, CA). The tester and driver double stranded cDNA were separately digested with five restriction enzymes (DraI, MscI, PvuII, SmaI, and StuI). These restriction enzymes generated blunt end cuts and the digestion resulted in an average insert size of 600 bp. Digestion with this set of restriction enzymes eliminates the step required to generate blunt ends by filling in of the cDNA ends. These modifications did not affect subtraction efficiency.

Eighty-five clones were isolated and sequenced. The determined cDNA sequences for 31 of these clones are provided in SEQ ID NO: 117-147. The sequences of SEQ ID NO: 122, 124, 126, 127, 130, 131, 133, 136, 139 and 147 showed no significant homologies to previously identified sequences. The sequences of SEQ ID NO: 120, 129,

135, 137, 140, 142, 144 and 145 showed some similarity to previously identified gene sequences, while the sequences of SEQ ID NO: 114, 118, 119, 121, 123, 125, 128, 132, 134, 138, 141, 143 and 147 showed some similarity to previously isolated ESTs.

In further studies, three additional cDNA libraries were generated from poly A+ RNA from a single small cell lung carcinoma sample subtracted against a pool of poly A+ RNA from nine normal tissues (lung, brain, kidney, liver, pancreas, skin, heart pituitary gland and spleen). For the first library (referred to as SCL2), the subtraction was carried out essentially as described above for the LAP1 library, with the exception that the tester and driver were digested with PvuII, StuI, MscI and DraI. The ratio of tester and driver cDNA used was as recommended by Clontech. For the second library (referred to as SCL3), subtraction was performed essentially as for SCL2 except that cDNA for highly redundant clones identified from the SCL2 library was included in the driver cDNA. Construction of the SCL4 library was performed essentially as described for the SCL3 library except that a higher ratio of driver to tester was employed.

Each library was characterized by DNA sequencing and database analyses. The determined cDNA sequence for 35 clones isolated from the SCL2 library are provided in SEQ ID NO: 245-279, with the determined cDNA sequences for 21 clones isolated from the SCL3 library and for 15 clones isolated from the SCL4 library being provided in SEQ ID NO: 280-300 and 301-315, respectively. The sequences of SEQ ID NO: 246, 254, 261, 262, 304, 309 and 311 showed no significant homologies to previously identified sequences. The sequence of SEQ ID NO: 245, 248, 255, 266, 270, 275, 280, 282, 283, 288-290, 292, 295, 301 and 303 showed some homology to previously isolated ESTs, while the sequences of SEQ ID NO: 247, 249-253, 256-260, 263-265, 267-269, 271-274, 276-279, 281, 284-287, 291, 293, 294, 296-300, 302, 305-308, 310 and 312-315 showed some homology to previously identified gene sequences.

3264 cDNA clones from three PCR-based subtracted cDNA libraries were analyzed by cDNA microarray technology as part of Lung Chip 5. Of the 3264 cDNA clones 960 clones came from SQL1 library, 768 clones came from SCL1 library, and 1536 clones came from SCL3 and SCL4 libraries. 35 pairs of fluorescent labeled cDNA probes

were used for the microarray analysis. Each probe pair included a lung tumor probe paired with a normal tissue probe. The expression data was analyzed. 498 cDNA clones were found to be overexpressed by 2-fold or greater in the small cell and/or non-small cell lung tumor probe groups compared to the normal tissue probe group. Also, the mean expression values for these clones in normal tissues were below 0.1 (range of expression is from 0.001 to 10). The cDNA sequences disclosed in SEQ ID NO:1240-1563 represent 324 non-redundant clones.

The following sequences were novel based on database analysis including GenBank and GeneSeq: SEQ ID NO:1240, 1243, 1247, 1269, 1272, 1280, 1283, 1285, 1286, 1289, 1300, 1309, 1318, 1319, 1327, 1335, 1339, 1346, 1359, 1369, 1370, 1371, 1393, 1398, 1405, 1408, 1413, 1414, 1417, 1422, 1429, 1432, 1435, 1436, 1438-1442, 1447, 1450, 1453, 1463, 1467, 1470, 1473, 1475, 1482, 1486, 1491-1494, 1501, 1505, 1506, 1514-1517, 1520, 1522, 1524, 1535, 1538, 1542, 1543, 1547, 1554, 1557, 1559, 1561, and 1563.

Full-length sequence for contig 139 (SEQ ID NO: 1467), also known as L985P, was identified by searching public databases using SEQ ID NO: 1467 as a query. By this approach, L985 was identified as cell surface immunomodulator-2 (CSIMM-2), the cDNA sequence of which is set forth in SEQ ID NO: 1676, encoding a protein having the sequence set forth in SEQ ID NO: 1677.

By microarray analysis, L985P was overexpressed by 2-fold or greater in the lung tumor probe groups compared to the normal tissue probe group. Moreover, the mean expression values for L985P in normal tissues was below 0.2 (range of expression was from 0.01 to 10). By real-time PCR analysis, greater than 40% of small cell lung carcinoma lung tumor samples tested had elevated L985P expression relative to normal lung tissue. Of those that displayed elevated L985P, the level of expression was greater than 3-fold higher than in normal lung tissue samples. Low or no expression of L985P was detected in an extensive panel of normal tissue RNAs. These findings for L985P support its use both as a diagnostic marker for detecting the presence of lung cancer in a patient and/or as an immunotherapeutic target for the treatment of lung cancer.

D. Isolation of cDNA Sequences from a Neuroendocrine Library using PCR-Based cDNA Library Subtraction

Using the modified PCR-based subtraction process, essentially as described above for the LAP1 subtracted library, a subtracted cDNA library (referred to as MLN1) was derived from a lung neuroendocrine carcinoma that had metastasized to the subcarinal lymph node, by subtraction with a panel of nine normal tissues, including normal lung, brain, kidney, liver, pancreas, skin, heart, lymph node and spleen.

Ninety-one individual clones were isolated and sequenced. The determined cDNA sequences for 58 of these clones are provided in SEQ ID NO: 147-222. The sequences of SEQ ID NO: 150, 151, 154, 157, 158, 159, 160, 163, 174, 175, 178, 186-190, 192, 193, 195-200, 208-210, 212-215 and 220 showed no significant homologies to previously identified sequences. The sequences of SEQ ID NO: 152, 155, 156, 161, 165, 166, 176, 179, 182, 184, 185, 191, 194, 221 and 222 showed some similarity to previously identified gene sequences, while the sequences of SEQ ID NO: 148, 149, 153, 164, 167-173, 177, 180, 181, 183, 201-207, 211 and 216-219 showed some similarity to previously isolated ESTs.

The determined cDNA sequences of an additional 442 clones isolated from the MLN1 library are provided in SEQ ID NO: 341-782. The determined cDNA sequences of an additional 11 clones isolated from the MLN1 library are provided in SEQ ID NO: 1654-1664.

E. Isolation of cDNA Sequences from a Squamous Cell Lung Carcinoma Library using PCR-Based cDNA Library Subtraction

A subtracted cDNA library for squamous cell lung carcinoma (referred to as SQL1) was prepared, essentially using the modified PCR-based subtraction process described above, except the tester and driver double stranded cDNA were separately digested with four restriction enzymes (DraI, MscI, PvuII and StuI) cDNA from a pool of two squamous cell lung carcinomas was subtracted against cDNA from a pool of 10 normal

tissues, including normal lung, brain, kidney, liver, pancreas, skin, heart, spleen, esophagus and trachea.

Seventy-four clones were isolated and sequenced. The determined cDNA sequences for 22 of these clones are provided in SEQ ID NO: 223-244. The sequence of
 5 SEQ ID NO: 241 showed no significant homologies to previously identified sequences. The sequences of SEQ ID NO: 223, 225, 232, 233, 235, 238, 239, 242 and 243 showed some similarity to previously identified gene sequences, while the sequences of SEQ ID NO: 224, 226-231, 234, 236, 237, 240, 241 and 244 showed some similarity to previously isolated ESTs.

10 The sequences of an additional 12 clones isolated during characterization of cDNA libraries prepared from lung tumor tissue are provided in SEQ ID NO: 813-824. Comparison of these sequences with those in the GenBank database and the GeneSeq DNA database revealed no significant homologies to previously identified sequences.

15

EXAMPLE 2

SYNTHESIS OF POLYPEPTIDES

Polypeptides may be synthesized on a Perkin Elmer/Applied Biosystems Division 430A peptide synthesizer using FMOC chemistry with HPTU (O-Benzotriazole-
 20 N,N,N',N'-tetramethyluronium hexafluorophosphate) activation. A Gly-Cys-Gly sequence may be attached to the amino terminus of the peptide to provide a method of conjugation, binding to an immobilized surface, or labeling of the peptide. Cleavage of the peptides from the solid support may be carried out using the following cleavage mixture: trifluoroacetic acid:ethanedithiol:thioanisole:water :phenol (40:1:2:2:3). After cleaving for
 25 2 hours, the peptides may be precipitated in cold methyl-t-butyl-ether. The peptide pellets may then be dissolved in water containing 0.1% trifluoroacetic acid (TFA) and lyophilized prior to purification by C18 reverse phase HPLC. A gradient of 0%-60% acetonitrile (containing 0.1% TFA) in water (containing 0.1% TFA) may be used to elute the peptides.

Following lyophilization of the pure fractions, the peptides may be characterized using electrospray or other types of mass spectrometry and by amino acid analysis.

EXAMPLE 3

5 PREPARATION OF ANTIBODIES AGAINST LUNG CANCER ANTIGENS

Polyclonal antibodies against the lung cancer antigen L773P (SEQ ID NO: 783) were prepared as follows.

Rabbits were immunized with recombinant protein expressed in and purified
10 from *E. coli* as described above. For the initial immunization, 400 µg of antigen combined with muramyl dipeptide (MDP) was injected subcutaneously (S.C.). Animals were boosted S.C. 4 weeks later with 200 µg of antigen mixed with incomplete Freund's Adjuvant (IFA). Subsequent boosts of 100 µg of antigen mixed with IFA were injected S.C. as necessary to induce high antibody titer responses. Serum bleeds from immunized rabbits were tested for
15 L773P-specific reactivity using ELISA assays with purified protein and showed strong reactivity to L733P. Polyclonal antibodies against L773P were affinity purified from high titer polyclonal sera using purified protein attached to a solid support.

EXAMPLE 4

20 PROTEIN EXPRESSION OF LUNG TUMOR-SPECIFIC ANTIGENS

Full-length L773P (amino acids 2-364 of SEQ ID NO: 783), with a 6X His Tag, were subcloned into the pPDM expression vector and transformed into either BL21 CodonPlus or BL21 pLysS host cells using standard techniques. High levels of expression
25 were observed in both cases. Similarly, the N-terminal portion of L773P (amino acids 2-71 of SEQ ID NO: 783; referred to as L773PA), with a 6X His tag were subcloned into the vector pPDM and transformed into BL21 CodonPlus host cells. Low levels of expression were observed by N-terminal sequencing. The sequence of the expressed constructs for L773P and L773PA are provided in SEQ ID NO: 784 and 785, respectively.

EXAMPLE 5

EXPRESSION IN E. COLI OF L548S HIS TAG FUSION PROTEIN

5 The L548S coding region was PCR amplified with the following primers:

Forward primer starting at amino acid 2:

PDM-433: 5' gctaaaggtgacccaagaaccaaag 3' Tm 60°C (SEQ ID NO:1665)

Reverse primer creating a XhoI site after the stop codon:

10 PDM-438: 5' ctattaactcgagggagacagataaacagtttcttta 3' Tm 61°C (SEQ ID NO:1666)

 The PCR product was then digested with XhoI restriction enzyme, gel
purified and then cloned into pPDM His, a modified pET28 vector with a His tag in frame,
which had been digested with Eco72I and XhoI restriction enzymes. The correct construct
15 was confirmed by DNA sequence analysis and then transformed into BL21 (DE3) pLys S
and BL21 (DE3) CodonPlus RIL expression hosts.

 The protein sequence of expressed recombinant L548S is shown in
SEQ ID NO:1667, and the DNA sequence of expressed recombinant L7548S is shown in
20 SEQ ID NO:1668.

 From the foregoing it will be appreciated that, although specific
embodiments of the invention have been described herein for purposes of illustration,
various modifications may be made without deviating from the spirit and scope of the
25 invention. Accordingly, the invention is not limited except as by the appended claims.